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STOCKHOLM 1957.

KUNGL. BOKTRYCKERIET P. A. NORSTEDT & SÖNER



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## **The Incorporation of Labelled Phosphate into Different Compounds in the Rat Kidney. The Effect of Parathyroid Extract on an *in vivo* System.**

By

**CARL-HENRIC DE VERDIER.**

Received 11 November 1956.

According to most investigators the renal tubule is the prime locus for the action of the parathyroid hormone (*e. g.* JACOBS and VERBANCK 1953, BARTTER 1954). In a recent article DAVIES, GORDON and MUSSETT (1956) have shown that parathyroid extract can be separated into one fraction which acts mainly on the excretion of phosphate by the kidney and another which mainly causes a liberation of bone salts.

During recent years there has been a tendency to localize the action of hormones to special chemical reactions (*e. g.* SUTHERLAND 1955). Concerning parathormone IMRIE and HELKINSON (1932) have observed that it accelerates the synthesis of creatine phosphate in cat's muscle, and quite recently NEUMAN, CHEN, FIRSCHEIN, MULRYAN and DI STEFANO (1956) have presented a theory, that the action of the hormone on the production of citrate in bone may be explained by a destruction of TPN<sup>1</sup>.

<sup>1</sup> Abbreviations are used as follows: AMP, ADP, ATP = adenosine mono-, di-, and triphosphate; UMP, UDP = uridine mono-, and diphosphate; GMP, GDP = guanine mono-, and diphosphate; CMP = cytidine monophosphate; IMP = inosine monophosphate; DPN and TPN = diphospho- and triphosphopyridine nucleotides; IP = inorganic orthophosphate; G-1-P and G-6-P = glycose-1-, and glucose-6-phosphate; F-6-P = fructose-6-phosphate; SerP and ThrP = serine-, and threonine-0-phosphate; TCA = trichloroacetic acid.

However, if the hormone mainly acts upon the renal excretion of phosphate it ought to influence some phosphorylation processes there. The observation of CARGILL and WITHAM (1949) that the parathyroid hormone lessens the absorption of glucose in the tubuli rather supports than contradicts such an assumption.

### Methods.

Two experiments have been performed. In the first one two groups of female rats were used with six animals in each group. The mean weight of the individuals was in the first group  $203 \pm 22$  g (S. D.) and in the second  $203 \pm 25$  g. The same rat stock was used as in earlier experiments on phosphoprotein metabolism (ÄGREN, DE VERDIER and GLOMSET 1954). The experiments were done in November and January in the morning and the animals had been fed *ad libitum* (TERPERMAN, L'HEUREUX and VILHELM 1947). One group was given injections of 0.2 U. S. P. Units of parathyroid extract (Parathormone, Eli Lilly and Comp.<sup>1</sup> Batch no. 1188—632610) per g body weight. The other group was given the same volume of physiological saline. After 30 min all animals were injected intraperitoneally with a dose of about  $3 \mu\text{C}$   $\text{P}^{32}$ -labelled phosphate per g body weight. 60 min after the injection of radiophosphorus the rats were killed by exsanguination through heart puncture under light ether anaesthesia. The kidneys were immediately removed and frozen in liquid air.

In the other experiment rats were used, which had been parathyroidectomized by electrocautery (e. g. MUNSON 1955). The mean weights for these groups were  $216 \pm 25$  g (hormone treated) and  $219 \pm 26$  g (controls). These animals were given an approximately double dose of radioactivity. In this experiment the livers were also collected, the conditions otherwise being identical. The separations and determinations of specific activity of the phosphocompounds mainly followed the methods earlier described (ÄGREN, DE VERDIER and GLOMSET 1954, 1956). Only Dowex 1 with 2 % DVB has been used as it gives quite as good separation as Dowex 1, 10 % DVB, while allowing elution with lower formate ion concentrations. The ammonium formate gradient was in some cases estimated by conductometric determinations of the concentration in different tubes. Free formic acid was removed from the tubes by lyophilization. If the fractions contained ammonium formate the ammonium ions were exchanged by rapid passage through a bed of Dowex 50, H-form.

To facilitate comparison the values for the specific activities given (table III) correspond to an isotope concentration in the injection solution of  $10^8$  cpm per ml. The injection solution contained in the first experiment  $1.64 \cdot 10^8$  cpm per ml; in the second one  $2.97 \cdot 10^8$  cpm per ml. Ultraviolet absorption was measured with a Beckman model

<sup>1</sup> The author is indebted to Eli Lilly and Comp. for the gift of the hormone preparation.

DU spectrophotometer. The identity of the nucleotides was checked by comparing absorption curves and by paper chromatography in solvents III and I (*Pabst Laboratories* 1956). Serum calcium was determined in the second experiment by a slight modification of FALC's method (1953), allowing 1 cm Beckman cells to be used.

### Results.

Before the hormone-treated group could be compared with the control group it was necessary to show that the rates of radio-phosphate absorption from the peritoneal cavity did not differ significantly. For that purpose repeated blood samples were taken from the tail vein of nembutal anaesthetized rats which had been treated as the other experimental animals. Fig. 1 shows how the isotope concentration varies with time.

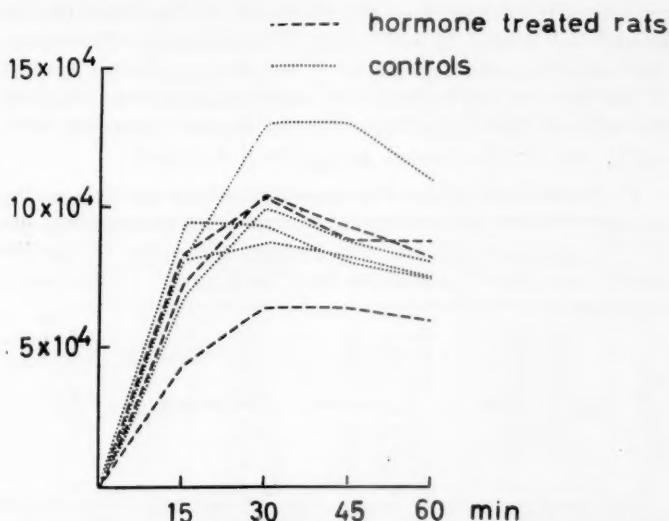


Fig. 1. The concentration of radioactivity in cpm per ml blood in samples taken at different times after  $P^{32}$  injection in nembutal anaesthetized female rats.

In the experiments total and inorganic phosphorus in heparin plasma was determined. These values and the distribution of the radioactivity are given in Table I. The total amount of radioactivity in the individual plasma samples showed a coefficient of variation of  $\pm 17.3\%$  for the control group and  $\pm 16.3\%$

Table I.

*The distribution of phosphorus and radioactivity in the plasma samples.*

	Normal rats		Ectomized rats	
	Control (N = 6)	Hormone treated (N = 6)	Control (N = 6)	Hormone treated (N = 6)
Total P, $\mu\text{g/ml}$ .....	144	128	1—	78
Total activity, cpm/ml .....	105,000	121,000	138,000	88,000
Spec. activity, cpm/ $\mu\text{g P}$ ....	735	944	—	1,139
Inorganic P, $\mu\text{g/ml}$ .....	52	45	60	34
Inorganic P, activity cpm/ml.	71,000	77,000	98,000	50,000
Spec. activity cpm/ $\mu\text{g P}$ .....	1,380	1,700	1,640	1,480

<sup>1</sup> The analysis failed.

for the hormone treated group. In the experiment with the parathyroidectomized rats the coefficient of variation for the groups was  $\pm 20.0\%$  and  $\pm 24.7\%$  respectively. That means that the difference is significant ( $P \approx 0.04$ ). As a further measure of the effect of the hormone the plasma samples were analysed for calcium. The values were for the control group  $9.8 \pm 1.0$  mg-% and for the treated group  $11.4 \pm 1.1$  mg-%.

*TCA-soluble substances.* The elution curves for the TCA-soluble substances were followed both by measuring radioactivity and UV-absorption at 260  $m\mu$ . Fig. 2 shows the curves for the first elution analysis of substances from both groups in the second experiment.

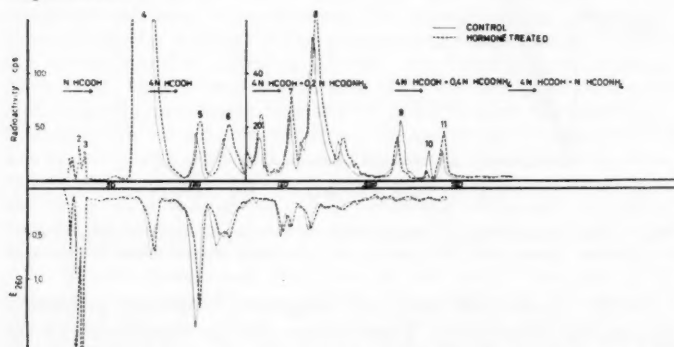


Fig. 2. Elution curves showing separation of TCA-soluble substances from parathyroidectomized rats. Peaks in positive direction indicate radioactivity, in negative direction UV-absorption. The arrows indicate changes in the storage flask concentration.

The values of the activities are plotted in the positive direction of the ordinate and the values of the absorption in the negative direction. The abscissa shows the tube number. A similar curve was obtained in the first experiment. The material eluted only with water is not included. The first peak is complex and contains CMP. Attempts to separate it further have not been made. Peak 2 and 3 correspond to DPN and AMP respectively. The fourth peak is mainly IP.

The ultraviolet absorbing material can with another Dowex 1 column and an ammonium formate gradient (HURLBERT, SCHMITZ, BRUMM and POTTER 1954) be separated from IP, which moves more quickly through the column. The main part behaved on the chromatogram strip as TPN. In experiments with pure G-6-P, G-1-P and F-6-P in the formic acid system they emerged in the latter part of IP. With paper chromatography and the solvents recommended by BANDURSKI and AXELROD (1951) for hexose phosphate, peak 4 did not contain any activity corresponding to sugar phosphates. Peak 5 gave by rechromatography three radioactive and UV-absorbing peaks (Table II). The first

Table II.

*Characteristics of some fractions from the TCA-extract.*

Fraction	Gradient of $\text{HCOONH}_4$ in M	$\lambda_{\text{max}}$ in $\text{m}\mu$	$R_f$ -value
5 A .....	0.3	261 (pH 4)	0.67 (III)
5 B .....	0.4	250 (pH 4)	0.53 (III)
5 C .....	0.5	260 (pH 4)	0.41 (III)
6 A .....	0.2	—	0.95 (III)
			0.72 (b. s.)
6 B .....	0.65	259 (pH 5)	0.28 (III)
7 .....	—	254 (pH 7)	0.51 (III)
		256 (pH 2)	
8 .....	0.45	262 (pH 5)	0.63 (III)

III = solvent III, (Pabst Laboratories 1956); b. s. = basic solvent (BANDURSKI & AXELROD 1951).

one is supposed to be UMP and the second IMP. The identity of the third (5 C) is unknown. Peak 6 contains mainly two substances, one (6 A) that is easily eluted with ammonium formate and is non-absorptive at 260  $\text{m}\mu$  and another that behaves as ADP. Peak 7 was not rechromatographed but moved as a single spot on the chromatogram. It is possibly identical with GDP. Peak 8 was the highest of the later eluted substances both with

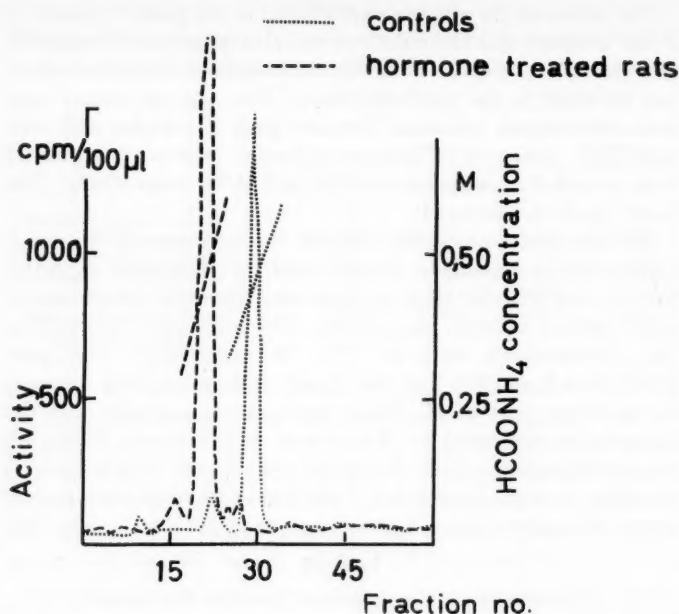


Fig. 3. Two parallel elution analyses by rechromatography of peak 8. Column: Dowex 1, formate form, 2 % cross-linkage,  $30 \times 1$  cm. The straight lines show the ammonium formate concentration.

regard to radioactivity and ultraviolet absorbency. Rechromatography gave only one high peak (Fig. 3).

The material gave an absorption curve accordant with one for a uridine nucleotide. The UV-absorbing spot on the paper chromatogram was elliptical and corresponded in position to UDP and UMP originating through decomposition. After hydrolysis with 0.01 N HCl (HURLBERT and POTTER 1954) no spot was obtained on the chromatogram reacting with the aniline phthalate reagent. These observations are compatible with the assumption that fraction 8 and UDP are identical.<sup>1</sup> Fraction 9—11 contained no or very small amounts of UV-absorbing material. Fraction 10 contained too little material to be analysed and fraction 11 was not homogeneous on the chromatogram strip.

<sup>1</sup> Attempts to isolate the substance from pig kidney are in progress.

Table III.

*Specific activity of different chromatographically purified substances in cpm per  $\mu\text{g}$  P (corrected for an injection solution containing  $10^8$  cpm per ml).*

	Normal rats		Ectomized rats	
	Control	Hormone treated	Control	Hormone treated
<b>From kidney</b>				
<i>TCA-soluble substances:</i>				
DPN .....	57	90	66	116
AMP .....	220	331	163	188
IP .....	380	584	852	988
TPN .....			633	286
UMP .....			155	173
IMP .....			187	602
Fraction 5 C .....			744	372
Fraction 6 A .....			424	592
ADP .....	377	440	440	574
Fraction 7 .....	530	610	309	780
Fraction 8 .....	570	808	459	1,530
Fraction 9 .....			346	334
<i>RNA-nucleotides</i>				
CMP .....	4.0	5.9	11.0	20.6
AM-2-P .....	6.0	7.2	13.4	19.9
AM-3-P .....			13.3	18.9
<i>Phosphorylated amino acids from phosphoproteins</i>				
SerP .....	109	449	199	789
ThrP .....			392	624
Phosphopeptide .....				
<b>From liver</b>				
<i>RNA-nucleotides</i>				
CMP .....			12.9	11.3
AMP .....			12.3	7.3
<i>Phosphoproteins</i>				
SerP .....			776	586
Phosphopeptide .....			900	723

*Phosphoproteins.* The acid hydrolysate of the rest protein fraction was run through a Dowex 50 column to separate IP and the organic phosphate. SerP and ThrP was isolated from a Dowex 1 column eluted with 0.5 N formic acid (DE VERDIER 1955). Owing to a technical accident the phosphoanalyses of the SerP-fraction from the second experiment failed.

*RNA.* The different nucleotides from RNA have about the same specific activity (for references see SMELLIE 1955). For

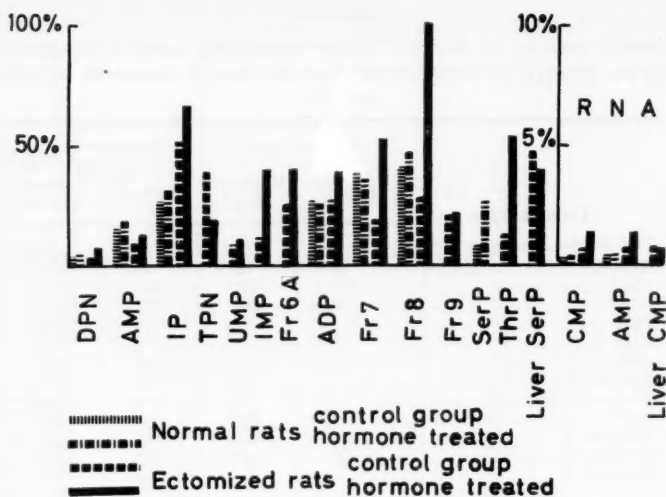


Fig. 4. Specific activities for different compounds expressed as *per cent* of the specific activity of plasma inorganic phosphate.

that reason only the most easily eluted CMP and AMP have been isolated.

The values of the specific activities are presented in Table III. In Fig. 3 the values are given as *per cent* of the specific activity of the inorganic phosphate in plasma.

### Discussion.

The methods for separation of nucleotides elaborated by HURLBERT et al. (1954) have considerably increased our possibilities to study phosphorus metabolism. The methods have mainly been used for liver tissue (HURLBERT et al. 1954 and BRUMM, POTTER and SIEKEVITZ 1956), liver mitochondria (SIEKEVITZ and POTTER 1955, BEYER, GLOMSET, and BEYER 1955), yeast cells (SCHMITZ 1954) and cancer cells (SCHMITZ, POTTER, HURLBERT and WHITE 1954) but not for kidney tissue according to the author's knowledge. The metabolism of phosphocompounds in the kidney in hyperglycemia has been studied by DRATZ and HANDLER (1952). They used older precipitation methods for the determination of radiophosphorus in different compounds. GER-

LACH (1954 and 1955) has combined precipitation methods and paper chromatography to determine the distribution of the total phosphorus in the kidney amongst different substances.

If the nucleotide pattern in liver given by HURLBERT et al. (1954) is compared with the pattern of the kidney, the main difference is the high peak 8 and the absence of the nucleoside triphosphates, particularly ATP. This is in contrast to the findings when the slices method is used to label the compounds in rat kidney *in vitro* (DE VERDIER, unpublished experiments). As high energy phosphate bonds are of importance at least for secretion in the tubuli (for references see EGGLETON 1956), one may speculate that they are rapidly used for that purpose. The morphological arrangement of the mitochondria toward the basal end of the cells does not contradict such an assumption. The main function of uridine diphosphate or its derivatives is hard to appraise. But as in other tissues it ought to play an important rôle in sugar transformations and detoxification (for references see HERBERT, POTTER and TAKAGI 1955). In that paper interrelations between adenosine and uridine nucleotides are also discussed. The incorporation rate of  $P^{32}$  into nucleotides of rat liver *in vivo* has been determined by BRUMM, POTTER and SIEKEVITZ (1956) and can be used for comparison.

The specific activities of the substances from the hormone treated animals are generally higher than those from non-treated groups. This is difficult to explain if one assumes that the parathyroid hormone acts solely on the absorption of phosphate in the tubuli. However, if there is a secretion of phosphate besides (KLEEMAN and COOKE 1951) or if the hormone causes a higher glomerular filtration rate (*e. g.* HANDLER, COHN and DE MARIA 1951) the finding is explicable. As could be expected the differences in specific activities between the groups are generally higher in the experiment with parathyroidectomized animals, and that seems not to be dependent on the blood level as reversed ratios are obtained for the compounds from the liver. If the groups not treated with hormone are taken together, it is remarkable that the specific activities for IP and RNA nucleotides are higher in the ectomized group. It is however in accordance to the results of TWEEDY, CHILCOTE, and PATRAS (1947), who studied the distribution of radiophosphorus between different organs in parathyroidectomized and parathormone treated rats.

Parathyroid extract seems to stimulate mostly the labelling

of fraction 8 and the phosphoproteins. For same substances it seems to lessen the degree of incorporation. The interpretation of this is difficult to give, but experiments are now in progress to ascertain whether differences also can be obtained in an *in vitro* system, *e. g.* with kidney slices. The high degree of labelling of the phosphoproteins is interesting and may be connected with the higher specific activity of phosphoproteins from the cell walls of *E. coli* incubated in radioactive phosphate (ÅGREN 1956), and the important function of phosphoprotein phosphorus, *e. g.* in hexokinase (ÅGREN and ENGSTRÖM 1956).

### Summary.

The incorporation of  $P^{32}$  into nucleotides, phosphoproteins and RNA from rat kidney has been investigated in normal and parathyroidectomized animals and the effect of parathyroid extract has been studied. The rats were killed 1.5 hours after the hormone injection and 1 hour after the parenteral administration of radioactive phosphate. The renal nucleotide pattern is characterized by a relatively high concentration of a substance which is most likely uridine diphosphate or a derivative of it. The parathyroid hormone seems to specially stimulate the incorporation of radiophosphate into this compound and into the phosphoproteins.

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## **Effects of Compound 48/80 on Blood Pressure and Plasma Histamine Level of Normal Dogs and Dogs with Mastocytoma.<sup>1</sup>**

By

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In 1949 BALTZLY, BUCK, DE BEER and WEBB prepared several substances with depressor effects one of which was called "compound 48/80". Chemically it was a condensation product of *p*-methoxyphenethyl-methylamine and formaldehyde, and, therefore, related to the large number of chemical compounds, *e. g.* ammonia, diamidines and diguanidines, which are active as histamine liberators. The pharmacological action of compound 48/80 was considered by PATON (1951), FELDBERG and TALESNIK (1953) to be the result of histamine liberation. In dogs and cats, PATON (*l. c.*) found that, simultaneously with a decrease in blood pressure, a release of histamine into the peripheral blood occurred together with a lengthening of the clotting time. The maximum release of histamine was reached after one minute, but the maximum increase in clotting time occurred 9 minutes after the administration of compound 48/80.

Several authors (MOTA, BERALDO and JUNQUEIRA 1953, FAWCETT 1954, RILEY and WEST 1955, BLOOM, LARSSON and SMITH 1956, BLOOM and LARSSON 1957) have found that compound 48/80 causes vacuolation and rupture of the mast cells in various animals. The effect of 48/80 on dog mastocytoma (mast cell tumours)

<sup>1</sup> The investigation was aided by a grant from the Swedish Cancer Foundation.

has not been investigated previously although the histamine content of these tumours has been found to be extremely high (RILEY and WEST 1953, CASS, RILEY, WEST, HEAD and STROUD 1954).

In Sweden mastocytomas occur chiefly in old boxers (LARSSON 1956). The high mast cell content of the tumours together with their accessibility and operability make them especially suitable for a study of the action of 48/80 on mast cells. The mast cells in these tumours, from a metabolic point of view, seem to correspond to normal tissue mast cells. That is, they contain both heparin (OLIVER, BLOOM and MANGIERI 1947, MAGNUSSON and LARSSON 1955) and histamine (RILEY 1952, 1953, RILEY and WEST 1953).

### Methods and Material.

Six clinically healthy, 1—7 years old, dogs of different breeds were used as controls. The dogs with mastocytoma consisted of seven boxers 8—11 years old and 3 boxers 7—9 years old which did not receive any 48/80. See table I.

Anaesthesia was induced with Intraval<sup>R</sup> (Pentobarbital sodium-U. S. P.) intravenously and maintained by inhalation of ether and oxygen. The blood pressure was recorded manometrically in mm of mercury through a catheter in the femoral artery. A silicone treated polyethylene catheter was inserted in the femoral vein for collection of blood samples. The samples were drawn into siliconed, heparinized tubes at 0, 1, 2, 5, 10 and 20 minutes respectively after the administration of compound 48/80. The tubes were immediately placed in an ice-bath and later centrifuged at 3,000 r. p. m. for five minutes. No hemolysis or only a very slight red tinge occurred. When the plasma had been pipetted off, it was immediately deep-frozen at  $-24^{\circ}\text{C}$  and held at this temperature until analyzed for histamine.

Compound 48/80 was administered intravenously into the V. saphena antebrachii usually in a dose of 0.1 mg per kg body-weight (table I.).

Histamine was determined in the plasma samples according to CODE's modification (1937) of the original method of BARSOUM and GADDUM (1935). This modification is particularly advantageous in the analysis of plasma (AHLMARK 1944).

To 4 ml plasma 8 ml of 10 per cent trichloroacetic acid was added. After one hour the protein-free filtrate was removed, and the protein washed 4 times with 3 ml of the acid. To the filtrate 8 ml of 10 per cent hydrochloric acid was added, and the mixture boiled for  $1\frac{1}{2}$  hours in a water bath. The mixture was dried by boiling *in vacuo* in a water bath, the residue treated with 10 ml of 96 per cent ethyl alcohol and dried again. This was repeated three times. The residue was then dissolved in 4 ml of Tyrode's solution and neutralized to pH 7 with sodium hydroxide.

Table I.  
*The Material.*

No. <sup>1</sup>	Dog no. <sup>2</sup>	Breed	Sex	Age years	Diagnosis	48/80 i. v. µg/kg	Determination performed of		
							Blood pres- sure	Histamine in plasma	tumour
—	I	Boxer	M	9	mastocytoma	100	—	+	—
1083/55	II	Boxer	F	10	mastocytoma	100	+	+	—
1080/55	III	Boxer	M	8	mastocytoma	100	+	+	+
—	IV	Swed. hound	M	3	healthy	100	+	+	—
81/56	V	Boxer	M	11	mastocytoma	30	+	+	+
—	VI	Boxer	M	7	healthy	100	+	+	—
—	VII	Mongrel	F	1	healthy	100	+	+	—
—	VIII	Chow-chow	M	5	healthy	100	+	+	—
560/56	IX	Boxer	F	9	mastocytoma	100	+	+	+
P 1543/56	X	Boxer	M	8	mastocytoma	100	+	—	+
—	XI	Spitz	F	1	healthy	100	+	+	—
—	XII	Mongrel	F	1	healthy	100	+	+	—
533/56	XIII	Boxer	M	8	mastocytoma	100	—	—	+
907/55	XIV	Boxer	F	7	mastocytoma	0	—	—	+
P 2858/55	XV	Boxer	F	9	mastocytoma	0	—	—	+
586/56	XVI	Boxer	F	7	mastocytoma	0	—	—	+

<sup>1</sup> The numbers refer to the patient numbers at the surgical clinic.

<sup>2</sup> These numbers refer to the latin numbers used throughout this article.

The samples were tested on guinea pig ileum in 2 ml aerated Tyrode's solution containing atropine sulphate (1 microgram per ml) at + 39° C.

*Histamine values are given as the free base.* The identity with histamine of the active substance was confirmed by the complete abolition of its action on guinea pig ileum by the addition of neoantergan (Anthisane<sup>®</sup>) in concentrations of  $10^{-6}$ — $10^{-7}$ .

The mastocytoma was removed surgically under general anaesthesia. When compound 48/80 had been administered, the tumour was removed after 30 minutes. A small piece of the tumour was removed for histological examination, while the remainder was analyzed for histamine.

Histamine determinations were performed both on a hydrochloric acid extract and on a trichloroacetic acid extract. For that reason the tumour was divided into two halves before the extractions were made. Each half was cut into small pieces and ground with sand with 5 ml of N 10 HCl and 2 ml of 10 per cent trichloroacetic acid per gram of tumour respectively. The HCl-mixture was heated until boiling for five minutes before filtering. The trichloroacetic acid extract was shaken at room temperature for 2 hours before filtration. In both cases the remains were carefully washed with the acid, the extracts neutralized with sodium hydroxide and histamine determined as described above.

When technically possible, the clotting time of the blood was determined before and after the administration of compound 48/80 (table III). The method of LEE and WHITE (1913) was employed using silicized glass-ware.

### Results.

1. The influence of compound 48/80 on the blood pressure is shown in fig. 1 & 2. *Healthy dogs:* The blood pressure fell, and the lowest value was reached within 2 minutes in 3 animals out of six. In the remaining 3 cases the blood pressure was not affected. In one of these cases (VI) another 0.1 mg per kg body-weight of compound 48/80 was injected intravenously after 10 minutes. This resulted in a blood pressure fall, but the minimum value of the other group was never reached. In all animals which reacted to the first injection, the blood pressure was returning toward the pre-injection value at the end of the observation period (20 minutes).

*Dogs with mastocytoma:* A precipitous fall in the blood pressure occurred in all dogs within a minute. The values recorded were considerably lower than the corresponding values in the healthy dogs. At the end of the observation period the pressure was still low in all cases. In case no. III no recording was done after ten minutes because of cardiac arrest. After heart massage the pressure returned to measurable values, but these values are not included. The blood pressure in dog no. I was not recorded because of a break-down of the manometer.

2. The effect of compound 48/80 on the histamine content of the blood plasma: The values found in *healthy dogs* are given in fig. 1. After 5 minutes a mean maximum value of 0.04  $\mu\text{g}$  of

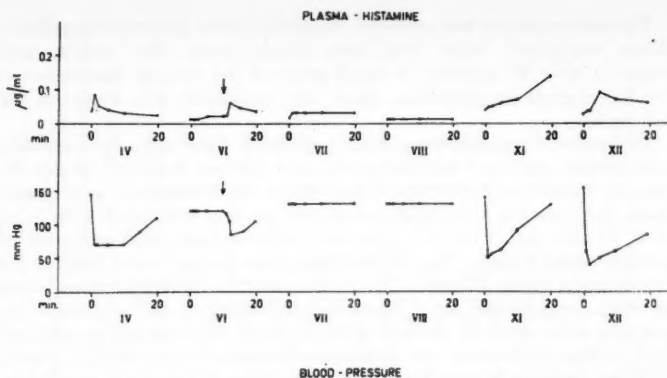


Fig. 1. Healthy dogs. At time 0 compound 48/80 was administered intravenously (0.1 mg/kg). Dog no. VI received another 0.1 mg/kg at time 10. Upper curve: plasma histamine (free base), lower curve: blood pressure.

histamine per ml of plasma was reached. This value was considerably lower than that found after 5 minutes in mastocytoma dogs where a value of 0.3  $\mu\text{g}$  per ml was recorded (fig. 2). Dog no. VI (healthy) did not respond to the first dose of 48/80 (0.1 mg/kg) and a second dose of 0.1 mg/kg was, therefore, given after 10 minutes. The histamine value rose after this second dose. Dog no. XI (healthy) showed increasing histamine values throughout the observation period. This was probably the result of inadequate anesthesia. Dog no. V (mastocytoma) only received 0.03 mg of compound 48/80 per kg body-weight which explains the low histamine-values, and the prompt return toward the normal level.

### 3. The effect of compound 48/80 on the clotting time:

A): Preinjection values: These were within normal values in all cases except one (II). In three cases (I, III, VIII) the values recorded were rather low.

B): After the administration of compound 48/80 a slight increase was noticed in three cases (III, V, VIII), a more prominent increase in one case (I), an enormous increase in one case (VI) and a decrease in one case (VII). The clotting time varied within the limits of the method in four cases (II, IV, IX, X).

4. The histamine content of mastocytoma: The values obtained are given in table II. The significance of the values is discussed below.

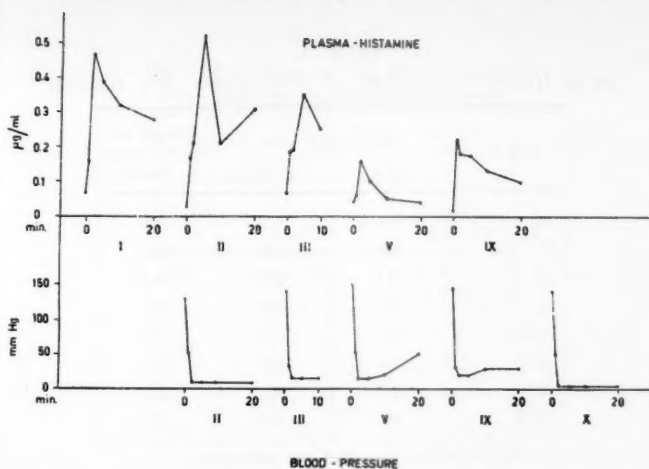


Fig. 2. Mastocytoma dogs. At time 0 compound 48/80 (0.1 mg/kg) was administered intravenously. Dog no. V only received 0.03 µg/kg.

Upper curve: plasma histamine (free base), lower curve: blood pressure.

### Discussion.

In earlier investigations by PATON (1951), FELDBERG and TALESNIK (1953), MOTA, BERALDO and JUNQUIERA (1953) and RILEY and WEST (1955) the dose of compound 48/80 administered was, as a rule, higher than in the present investigation. The fall in the blood pressure recorded by these authors in normal dogs was greater than in the present material, and when a new dose was administered, no further effect on the blood pressure was obtained. In the present investigation, however, a second similar dose of compound 48/80 administered after 10 minutes caused a further fall in the pressure (dog no. VI). This discrepancy is probably a result of the fact that the larger doses of histamine liberators deplete the stores of the histamine which can be liberated. The small doses utilized in the present investigation were chosen to enhance the differences between healthy dogs and dogs with mastocytoma. This difference was also clearly shown when the blood plasma was analyzed for histamine. The mastocytoma dogs had a mean plasma concentration about nine times that of healthy dogs. In neither the normal or mastocytoma dogs in this study was the maximum value of 2.2 micrograms per ml plasma recorded by PATON (1951) attained.

Table II.

*µg of Histamine per Gram of Mastocytoma (Wet Weight).*

Dog no.	Extraction with		Weight of mastocytoma in grams
	HCl	CCl <sub>3</sub> COOH	
III .....	114	15	24.2
V .....	428	229	46.4
IX .....	411	755	16.3
X .....	270	296	25.7
XIII .....	903	873	11.1
XIV .....	156	465	15.7
XV .....	683	681	10.2
XVI .....	— <sup>1</sup>	260	40.9

<sup>1</sup> No extraction with HCl was performed.

In the present investigation, the blood pressure of the normal dogs returned toward normal values within 30 minutes. In the experiments of PATON (l. c.) the values were lower and had not returned to normal within one hour. Therefore, the magnitude of the dose of compound 48/80 determines the immediate fall in blood pressure and the time lag before it returns to a normal level. In the dogs with mastocytoma this time lag coincides with that found by PATON (l. c.) although the dose administered was ten times smaller. One possible explanation of this result is a considerable release of histamine from the tumour. BALTZLEY, BUCK, DE BEER and WEBB (1949) employed the same dose of compound 48/80 as was used by the present author. In their experiments the maximal fall in blood pressure was not reached until 8—9 minutes. The magnitude of the fall recorded by these authors coincides with that found in the present investigation after two minutes. Furthermore, the form of the blood pressure curve in the experiments of BALTZLEY et al. did not coincide with that found by the present author. This might be due to differences in anaesthesia level and differences in purity of the compound 48/80. There are, however, great differences in sensitivity between different individuals (compare *e. g.* dogs no. VI & VIII with the rest of the animals in the present investigation). This variation among individuals was pointed out by BALTZLEY et al. (l. c.) and stressed by ARVY (1956). MACINTOSH and PATON (1949) in

Table III.

*The Clotting Time of the Blood Samples.*

Dog no.	Time after 48/80	Clotting time
I .....	0 m 13 m	3 m 00 s 14 m 00 s
II .....	0 m 7 m 20 m	10 m 00 s 8 m 30 s 9 m 30 s
III .....	0 m 6 m 30 s 90 m	2 m 30 s 4 m 15 s 6 m 00 s
IV .....	0 m 11 m 16 m	4 m 10 s 4 m 00 s 6 m 00 s
V .....	0 m 20 m	6 m 00 s 10 m 00 s
VI .....	0 m 5 m 30 m	5 m 00 s > 30 m > 30 m
VII .....	0 m 5 m 10 m	5 m 00 s 3 m 00 s 2 m 30 s
VIII .....	0 m 10 m 30 m	2 m 00 s 4 m 15 s 7 m 40 s
IX .....	0 m 10 m 28 m	7 m 00 s 10 m 00 s 10 m 00 s
X .....	0 m 25 m 32 m	5 m 00 s 4 m 00 s 4 m 00 s

m = minutes

s = seconds

addition, have shown that some individuals need fairly high doses of histamine liberators before they react.

PATON (1951) found that the clotting time of the blood was delayed after the administration of compound 48/80. This prolongation was most prominent 9 minutes after the drug had been

administered. The clotting time returned to normal slower than the plasma content of histamine.

Considerable variations in the clotting time of different individuals were found in the present investigation. The cause of this is not known at present. The results might, in general, be interpreted as a tendency toward a delayed clotting time. Such a tendency has been noted by QUIVY (1956). The enormous increase in dog no. VI is interesting but cannot be explained by the plasma histamine content. The discrepancies between the present results and those of QUIVY (1956) on the one hand and those of PATON (1951) on the other obviously need further explanation.

Although the histamine in the tumours was extracted with hydrochloric acid and trichloroacetic acid in parallel, it can not be stated which of the extraction methods is best (table II). There was no significant difference in histamine content between tumours from dogs treated or untreated with 48/80. This result is partly a function of the varying number of mast cells per gram of tumour. The highest histamine content in a mast cell tumour recorded in the literature is 1,290  $\mu\text{g}$  per gram of tumour (CASS et al. 1954). In the present investigation a maximal value of 903  $\mu\text{g}$  per gram of tumour (wet weight) was found. This value was found after the administration of 48/80. It is clear that mastocytoma of dogs contain large amounts of histamine, but differences in mast cell content between tumours and within a tumour make a comparison of values meaningless. In order to make such a comparison it would be necessary to do several biopsies before and after the administration of 48/80. A biopsy might, however, cause liberation of histamine because of the heavy bleeding (BLOOM, FRIBERG, LARSSON and ÅBERG 1955) which ruptures the tumour tissues in the vicinity of the biopsy site.

That histamine could be extracted in large amounts from tumours from dogs treated with a histamine liberator is probably an indirect result of the rapid fall in the blood pressure which slows the circulation. This, in turn, probably makes it impossible for the whole dose of compound 48/80 to reach the tumour. That some of it reaches the tumour is clear from histological studies which show that many mast cells are ruptured (BLOOM and LARSSON 1957). It must be stressed, however, that there is the possibility that old mastocytoma boxers have a generalized increase of mast cells throughout their bodies, and that these

cells not included in recognizable tumours are more or less responsible for the increased histamine levels found in the blood plasma after the administration of compound 48/80. On the other hand the present results do not exclude the possibility that the impaired circulation is the cause of the high amounts of histamine which are extractable from the tumour, *i.e.* that compound 48/80 reaches the tumour, liberates histamine, causes a marked fall in blood pressure and prevents the histamine from leaving the tumour.

### Summary.

Six healthy dogs and ten dogs with mastocytoma were studied. Of the latter, seven were treated with compound 48/80 intravenously. The blood pressure, plasma histamine and/or histamine content of the tumour was studied, and the results discussed in relation to the pertinent literature.

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## Food Intake and Oxygen Consumption in Pigeons at Low Temperatures.

By

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The metabolic cost of maintaining constant body temperature at various ambient temperatures has been measured in a variety of warm-blooded animals. Down to a certain point, designated as the critical temperature, the animal may remain at a basal heat production by increasing its insulation. Below this point, insulation remains at a maximum and heat loss follows essentially Newton's law of cooling, *i. e.* the heat production increases roughly linearly with decreasing temperatures (SCHOLANDER, HOCK, WALTERS, JOHNSON and IRVING, 1950). In some cases, for instance in wild Norway rats, this relation held up to an oxygen consumption six to seven times the resting rate (KROG, MONSON and IRVING, 1955). Most small arctic mammals and birds have a critical temperature well above 0° C. Many of them, especially the birds, do not seek shelter, but are fully exposed to the winter cold, and are forced to maintain a high metabolic rate for months at a time. One might expect that under conditions where weight, insulation and activity remain constant, food intake during long periods of cold stress would reflect Newton's law of cooling in the same way as oxygen consumption does in experiments of short duration.

It is well known that animals and man use more food when exposed to low temperatures (BROBECK 1948, STREICHER, HACKEL and FLEISCHMAN, 1950, *cp.* BURTON and EDHOLM 1955), but these investigations failed to reveal any simple relation between

the food intake and the cold stress. In the present investigation homing pigeons were kept at various temperatures long enough to produce a steady state equilibrium between food intake and air temperature, and the critical temperatures for the food intake and the oxygen consumption have been compared.

### Experimental.

The experimental procedure was briefly the following: Five adult homing pigeons were kept in individual wire cages, and acclimated for 2 to 4 weeks to  $-10^{\circ}\text{C}$ ,  $+18^{\circ}\text{C}$ , and  $-24^{\circ}\text{C}$ . The amount of food consumption was measured daily, and at the end of each period the critical temperature for  $\text{O}_2$  consumption was determined.

Activity was kept at a minimum by minimizing the diurnal light period. Pigeons eat only when it is light, and a seismograph arrangement showed that they always sit very quietly in the dark. They were therefore provided with only enough light to maintain their body weights. A regime with 2 hours' light at  $+18^{\circ}\text{C}$ , 10 to 12 hours' light at  $-10^{\circ}\text{C}$ , and 20 to 22 hours' light at  $-24^{\circ}\text{C}$  was found suitable. The weights of the pigeons were 370, 385, 385, 425, and 450 g, and varied no more than  $\pm 10$  g during the experimental period.

Rectal temperatures were measured with either thermocouples or a Hg thermometer, inserted 5 cm into the rectum. Skin temperatures on the breast were obtained by taping thermocouples on the naked skin under the feathers. Temperatures were measured to within  $\pm 0.1^{\circ}\text{C}$ .

Heat production was determined indirectly by means of the oxygen consumption, using an open circuit apparatus similar to that described by SCHOLANDER et al. (1950). The experiments started in the morning and lasted 9 to 12 hours. The pigeons were left for at least one hour at the desired temperature before sampling started. At each temperature three 10-minute samples of air were collected in a spirometer at intervals of one half hour, and analyzed for  $\text{O}_2$  and  $\text{CO}_2$  in the  $\frac{1}{2}$  cc gas analyzer (SCHOLANDER, 1947). Air flow was regulated to give from 0.50 to 1.00 per cent  $\text{CO}_2$  in the animal chamber. The temperature inside the respiration chamber was held within  $1^{\circ}\text{C}$  over a range from  $-35^{\circ}\text{C}$  to  $+30^{\circ}\text{C}$ .

Between runs, food was always available to the pigeons, and consisted of a mixture of three fourths whole corn, one fourth peas, and some durra. Daily food intake was measured each evening. The pigeons were provided with water three times a day. The total amount of dried faeces was weighed after the  $+18^{\circ}$  period and the  $-24^{\circ}$  period.

### Results.

*Body Temperatures.* Rectal temperature remained between  $41^{\circ}\text{C}$  and  $42^{\circ}\text{C}$ . It was about one degree lower during dark than

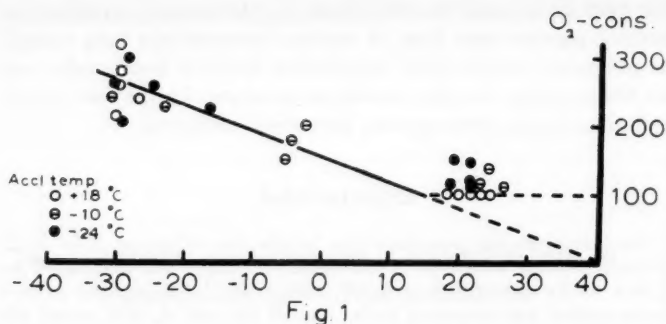


Fig. 1. Oxygen consumption of pigeons kept at various ambient temperatures. Each point represents the average of three determinations. Resting metabolic rate for each pigeon, taken at the end of the  $+18^{\circ}\text{C}$  period, is called 100, and the other values are given as per cent of this. The critical temperature is about  $14^{\circ}\text{C}$  and the diagonal line conforms with Newton's law of cooling.

during light periods, but was unaffected by the ambient temperature. Also the skin temperature remained practically constant throughout the experiments, namely about  $36^{\circ}\text{C}$  at  $18^{\circ}\text{C}$ , and  $34^{\circ}\text{C}$  at  $-30^{\circ}\text{C}$ .

*Oxygen Consumption.* It will be seen from Fig. 1 that the oxygen consumption below the critical temperature ( $+14^{\circ}\text{C}$ ) increases roughly linearly with the body-to-air gradient, reflecting Newton's law of cooling, and confirming earlier data on pigeons and other birds (cp. SCHOLANDER et al. 1950). There were no signs of moulting during the experiments, and the results in general indicate that the overall insulation remained constant. A slight elevation of the resting rate is apparent in the cold adapted animals. The respiratory quotient was found to be between 0.7 and 1.0, and showed no regular variation with ambient temperature. Similar results on pigeons and other animals have been reported by KAYSER (1937) and by IRVING et al. (1955).

*Food Intake.* The pigeons needed from two to ten days to reach a steady state equilibrium between food intake and air temperature. After this time the daily food intake varied by only  $\pm 10\%$ . The data presented are from such stable periods. In all periods the pigeons consumed the same assortment of food, and the faeces weights remained constant, i. e.  $21 (\pm 3)$  per cent of the food intake.

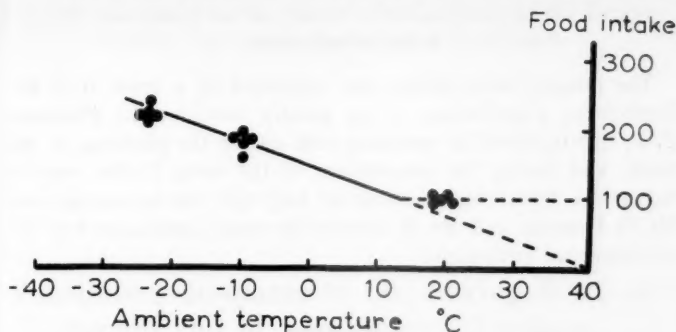


Fig 2

Fig. 2. Food intake compared to ambient temperature. Each plot represents the average daily food intake for one individual during a period of at least 8 days. Resting food intake for each pigeon during the  $+18^{\circ}\text{C}$  period is called 100, and other values are given in per cent of this. The critical temperature is about  $13^{\circ}\text{C}$ , and the diagonal conforms with Newton's law of cooling.

It is seen from Fig. 2 that the food intake below the critical temperature increased in fairly linear proportion to the body-to-air temperature gradient, reflecting Newton's law of cooling. The critical temperature for the food intake is about  $+13^{\circ}\text{C}$ , *i. e.* practically the same as the value found for oxygen consumption.

### Conclusion.

This investigation demonstrates that the food intake of pigeons exposed to cold below their critical temperature was closely proportional to the body-to-air gradient, and was hence accurately adjusted to the thermal needs of the animal.

### Summary.

The daily food intake has been measured in five pigeons while they were kept for 2—4 weeks at  $-10^{\circ}\text{C}$ ,  $+18^{\circ}\text{C}$ , and  $-24^{\circ}\text{C}$ . At the end of each period the oxygen consumption was measured at various temperatures between  $-35^{\circ}\text{C}$  and  $+20^{\circ}\text{C}$ . It was found that the food intake was closely correlated with the oxygen consumption, both of them indicating nearly the same critical temperature. Below this temperature both oxygen consumption and food intake increased linearly with the body-to-air gradient.

### Acknowledgement.

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## Ventilatory Response to $\text{CO}_2$ During Work at Normal and at Low Oxygen Tensions.

By

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The purpose of the present investigation is to study the sensitivity of the respiratory centre towards  $\text{CO}_2$  during muscular work at normal and at low alveolar  $\text{O}_2$  tension. The experiments were performed on normal human subjects working on the KROGH bicycle ergometer or on a motordriven treadmill. In the low oxygen experiments the oxygen tension of the inspired air was varied so as to keep the alveolar  $\text{pO}_2$  at a nearly constant value.

### Methods and Procedure.

The subjects came fasting to the laboratory in the morning. The experiments were performed in the steady state of work and usually two determinations in ordinary air preceded one or two periods of breathing various air mixtures in each of which four determinations were made. Enough time (8 to 12 minutes) elapsed before and between each series of determinations to secure that an equilibrium practically had been reached. The technique for the breathing of the air mixtures and for the adjustment of the oxygen tension of the inspired air was the same as described by NIELSEN and SMITH (1951). Considering the very high ventilations in the present experiments especially large valves and smooth-walled rubber tubings of inner diameter of 32 mm were used. Alveolar gas tensions were calculated from the BOHR formula using the dead spaces for  $\text{CO}_2$  as determined on normal young men in rest and during work by ASMUSSEN and NIELSEN (1956). Since this procedure can be used with safety only when the tidal air exceeds

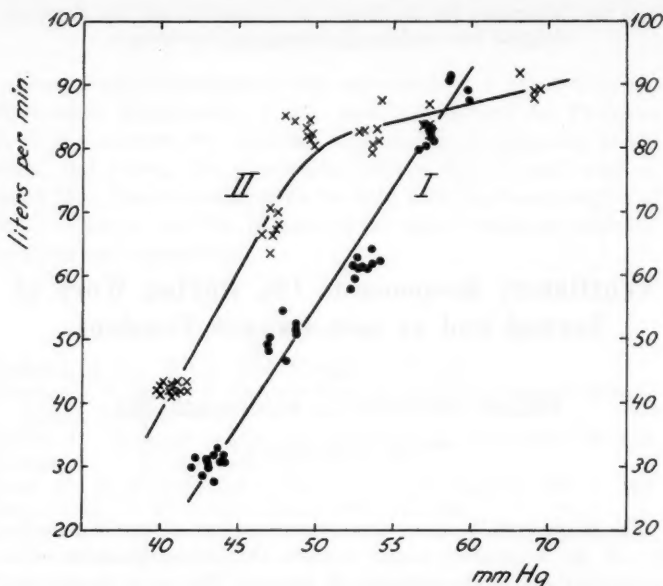


Fig. 1. Pulmonary ventilation (BTPS) in relation to alveolar  $p\text{CO}_2$ .  
Bicycle experiments. Subject E. A.

I 540 mkg/min.

II 720 " "

about 1.5 liter only experiments in which this was the case were considered in the present study. In a series of rest experiments also samples of end expiratory alveolar air were collected by the procedure described by NIELSEN and SMITH (1951). In 14 paired determinations it was found that the mean difference between the calculated and the directly determined  $\text{CO}_2$  tensions was  $-0.3$  mm Hg with a standard deviation of  $\pm 0.8$  mm Hg. As subjects served P. B. (24 years, 78.5 kg, 179 cm), K. S. (24 years, 71 kg, 178 cm) and E. A. (48 years, 67 kg, 171 cm).

### Results.

Fig. 1 shows the pulmonary ventilation in relation to the alveolar  $p\text{CO}_2$  at two grades of work (540 mkg/min. and 720 mkg/min.) performed at the bicycle ergometer with subject E. A. breathing mixtures of  $\text{CO}_2$  and atmospheric air. At the lower work intensity the ventilation increases linearly with increasing alveolar  $p\text{CO}_2$  throughout the whole range of determinations. At the higher

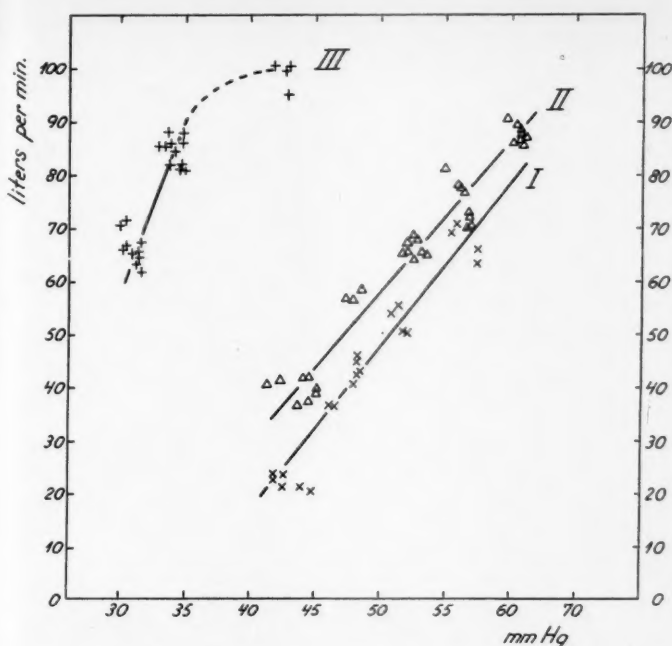


Fig. 2. Pulmonary ventilation in relation to alveolar pCO<sub>2</sub>.  
Treadmill experiments. Subject K. S.

- I CO<sub>2</sub>-air mixtures. Oxygen consumption 1.1 liters per min.  
 II CO<sub>2</sub>-air mixtures. Oxygen consumption 1.9 liters per min.  
 III CO<sub>2</sub>-low O<sub>2</sub> mixtures (alveolar pO<sub>2</sub> 53.2 mm Hg, range 51.0–55.2 mm Hg). Oxygen consumption 2.0 liters per min.

work intensity the ventilation also showed a practically linear increase up to an alveolar pCO<sub>2</sub> of about 50 mm Hg but at higher alveolar CO<sub>2</sub> tensions (50 to 64 mm Hg) the ventilation only underwent a relatively very small increase. The rectilinear part of the curve is displaced to the left of the curve for the lower work intensity and shows a somewhat increased steepness. In bicycle experiments performed on the other two subjects curves of principally the same form as those described above were found.

As the subjects in the bicycle experiments were inclined to adapt their frequency of breathing to the rhythm of work, which was set by a metronome, and as it was found, that the relation between ventilation and alveolar pCO<sub>2</sub> at high ventilations was

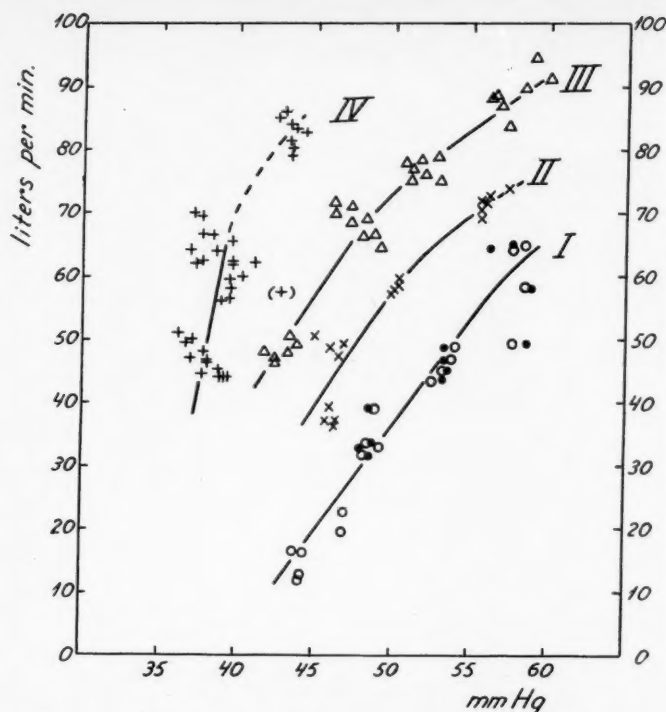


Fig. 3. Pulmonary ventilation (BTPS) in relation to alveolar  $p\text{CO}_2$ .

Rest and treadmill experiments. Subject P. B.

I Rest experiments.  $\text{CO}_2$ -air mixtures.  $\bullet$ — $\bullet$   $p\text{CO}_2$  values calculated.  $\circ$ — $\circ$   $p\text{CO}_2$  end tidal values.

II and III Treadmill experiments.  $\text{CO}_2$ -air mixtures. Oxygen consumption 1.1 liter per min. and 2.1 liters per min. respectively.

IV Treadmill experiments.  $\text{CO}_2$  low  $\text{O}_2$  mixtures. Alveolar  $p\text{O}_2$  54.8 mm Hg, range 52.1–58.2 mm Hg. Oxygen consumption 1.8 liter per min.

influenced by the respiratory frequency and depth chosen by the subject, it was decided to perform further experiments on the treadmill where the rhythm of work is not fixed.

In fig. 2 and fig. 3 such experiments are presented together with the experiments in low oxygen and a series of rest experiments (only on subject P. B.). Fig. 2 (subject K. S.) shows that the two stimulus-response curves from the two series of experiments performed in atmospheric air are linear in the whole range

Table 1.

Subject	Condition	O <sub>2</sub> -uptake l/min.	"Sensitivity index" <sup>1</sup> l/mm Hg	
			air	low O <sub>2</sub>
E. A.	Bicycling	0.8	3.0	
	"	1.4	3.7	
	"	1.8	4.5	
P. B.	Resting	0.28	3.5	
	Bicycling	1.4	3.8	
	"	1.8	3.6	
	"	2.5	2.5	
	"	3.2	3.6	
	Walking, horiz.	1.1	3.8	
	" , uphill	1.8	—	13.0
	" , "	2.1	3.9	
K. S.	Bicycling	1.5	3.4	
	"	1.8	5.2	
	"	2.5	3.3	
	"	3.2	(5.0)	
	Walking, horiz.	1.1	3.1	
	" , uphill	1.9	2.9	
	" , "	2.0	—	6.5

<sup>1</sup> Increase in ventilation per mm increase in alveolar pCO<sub>2</sub>.

and are very nearly parallel; the curve for the heavier work is shifted somewhat to the left. In the corresponding experiments on subject P. B. (fig. 3) the experiments in which no CO<sub>2</sub> was added to the inspired air are omitted because the respiratory depth was so low (below about 1.5 liter) that the alveolar pCO<sub>2</sub> could not be determined with sufficient accuracy. The lower parts of the stimulus-response curves are therefore less well defined, but seem to be nearly parallel to each other and to the curve from the resting experiments. The upper part of the curves from the work experiments shows a distinct decrease of steepness.

The stimulus-response curves from the experiments in low oxygen (fig. 2 and fig. 3, alveolar pO<sub>2</sub> 53.2 mm Hg and 54.8 mm Hg) are displaced to the left of and are much steeper than the curves from the work experiments at normal pO<sub>2</sub> at the same oxygen uptake. The upper parts of the curves show a decreasing steepness already at relatively low values of the alveolar pCO<sub>2</sub>.

The steepness of the stimulus-response curves is a measure of the sensitivity of the respiratory center to  $\text{CO}_2$  and can be expressed by the "sensitivity index" *i. e.* the increase in ventilation per mm Hg increase in alveolar  $\text{pCO}_2$ . Table 1 shows the "sensitivity indices" from all the series of experiments performed. It appears from the table, that in subject E. A. the "sensitivity index" increases somewhat with increasing severity of work. In P. B. the "sensitivity index" seems to be practically constant at rest and during work at normal  $\text{pO}_2$  with one inexplicable exception at an oxygen uptake of 2.5 liters per minute. Also in subject K. S. — with two exceptions one of which is based on only one  $\text{CO}_2$  breathing experiment (4 determinations) — the "sensitivity index" is practically constant in the air experiments. In the low oxygen experiments the "sensitivity index" is both in subject K. S. and P. B. considerably higher than in the corresponding air experiments.

### Discussion.

The present experiments show that during work the respiratory center reacts to increase in the alveolar  $\text{pCO}_2$  in much the same way as during rest, *i. e.* the ventilation increases linearly up to a certain value with increasing values of  $\text{pCO}_2$ , but the stimulus-response curves are displaced to the left of the resting curve and the more so the higher the work intensity. Threshold values obtained by rectilinear extrapolation of the work curves are therefore correspondingly lowered. The steepness of the stimulus-response curves ("sensitivity index") is in the majority of the experiments of about the same magnitude and only one subject showed a tendency to an increase in steepness with increasing work intensity. The results from these experiments are in agreement with the results of NIELSEN (1936 a), but do not lend support to the conclusions drawn by CRAIG (1955), viz. that the curves relating ventilation to carbon dioxide tension are exponential curves with increasing slope and that the two factors work and carbon dioxide operate so as to diminish the action of one another. CRAIG, however, relates the ventilatory response to the  $\text{CO}_2$  tension of the inspired air and from such a relation no conclusions can be drawn as to the influence of  $\text{CO}_2$  on the regulation of the respiration. If for instance in the present experiments the ventilation had been related to the inspiratory  $\text{CO}_2$  tension ex-

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ponential curves similar to those of CRAIG would have been obtained.

In the low oxygen experiments the lower part of the stimulus-response curves is much steeper than and displaced to the left of the corresponding curves from the experiments in atmospheric air at the same work intensity. In rest experiments with acute severe hypoxia it has also been found (NIELSEN and SMITH 1951, HALL 1953 and LLOYD, JUKES and CUNNINGHAM 1956) that the stimulus-response curve is much steeper than normally but in contradistinction to the work experiments it was found that this powerful CO<sub>2</sub> effect was only apparent above a certain value of the alveolar pCO<sub>2</sub> which was close to the threshold value of CO<sub>2</sub> in normal air. Lower CO<sub>2</sub> tensions in the interval from the threshold value down to the values obtaining in the experiments in which no CO<sub>2</sub> was added to the inspired air (basic experiments) had only a slight effect on the ventilatory response. That the ventilation during work in low oxygen increases already at a very small increase in alveolar pCO<sub>2</sub> produced by adding CO<sub>2</sub> to the inspired air may be explained either by the fact that the alveolar pCO<sub>2</sub> already in the basic experiments (no CO<sub>2</sub> added) is higher than the extrapolated threshold value for the same condition of work in normal air or by the assumption that the threshold value during work in low oxygen is lowered. The reason for such a lowering of the threshold might be that the metabolism during these experiments with work in low oxygen doubtless is markedly anaerobic in contradistinction to the metabolism at rest which even in severe hypoxia shows no measurable signs of anaerobic conditions.

As mentioned earlier the stimulus-response curves in the upper ranges of alveolar CO<sub>2</sub> tensions have a tendency to deviate towards the abscissa (see fig. 1). This flattening-out of the curves takes place at a lower CO<sub>2</sub> tension when the work intensity is higher, and if work is performed in low oxygen it seems to occur at a still lower CO<sub>2</sub> tension. Corresponding results were observed by NIELSEN (1936, a and b). Large individual differences seem to exist as to the CO<sub>2</sub> tension at which the deviation takes place at a given intensity of work. It might be thought that the cause of the deviation is that the subject is approaching the limit of his breathing capacity. This assumption is unlikely for several reasons: first, the deviation sometimes takes place at a ventilatory rate much lower than attainable at higher work intensities;

second, in spite of the large increase in alveolar  $p\text{CO}_2$  with only an insignificant increase in ventilation no accompanying air hunger is felt.

Another explanation may be that  $\text{CO}_2$  at higher concentrations has an inhibitory effect on the respiratory center. This effect may partly be due to the well known narcotic effect of  $\text{CO}_2$  in high concentrations. But the main reason is probably that  $\text{CO}_2$  in high concentrations exerts a special depressant effect on the respiratory center. This assumption is supported by the fact that the deviation of the stimulus-response curves can take place at a much lower  $\text{CO}_2$  tension in work than at rest. In addition the ventilation at rest at inspiratory  $\text{CO}_2$  concentrations above 9 per cent may again decrease and in some cases even has been observed to stop completely after a transitory violent hyperpnoea without any loss of consciousness (NIELSEN 1936 a and b and unpublished data). Finally during work in low oxygen this depressant effect of high  $\text{CO}_2$  concentrations on the ventilation seems to occur at a still lower alveolar  $p\text{CO}_2$  than during work in ordinary air (see fig. 2 and fig. 3).

### Summary.

The sensitivity of the respiratory center to  $\text{CO}_2$  during muscular work at normal and at low oxygen tensions has been studied in three human subjects.

It was found that the rectilinear part of the stimulus-response curves in normal air was displaced to the left of the resting curve and the more so the higher the work intensity. The steepness of the curves was in the majority of cases about the same. In low oxygen the stimulus-response curves were displaced more to the left and were much steeper than for the same work intensity in normal air. The flattening of the stimulus-response curves found at higher  $\text{CO}_2$  concentrations is assumed to be due mainly to a special depressant effect of high  $\text{CO}_2$  tensions on the respiratory center.

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## Heparin Monosulphuric Acid, $\beta$ -Heparin and Dog Mast Cell Tumour Heparin in Different Clotting Systems.

By

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It is known that heparins from various sources differ in anti-coagulant potency and mode of action. JAKES and co-workers (JAKES 1940, JAKES and CHARLES 1941, JAKES, WATERS and CHARLES 1942, JAKES, BELL and CHO 1954) for example found the biologic activity of dog heparin to be two and a half times that of ox heparin which was, in turn, five times as potent as sheep heparin. These authors used cat whole blood for the anti-coagulant assay. MARBET and WINTERSTEIN (1952), who compared the antithromboplastin and antithrombin activity of various heparins, found the antithrombin activity of dog heparin relative to ox heparin to be identical with that for the antithromboplastin activity.  $\beta$ -heparin from ox lung and from sheep lung, on the other hand, showed a considerably lower antithromboplastin activity in relation to their antithrombin activity. YAMASHINA (1954) pointed out that the high activity of  $\beta$ -heparin in thrombin systems could not be demonstrated in assays with whole blood or recalcified bovine plasma or *in vivo* in cats or dogs. The heparin monosulphuric acid behaved like ordinary ox heparin, the antithrombin activity being of about the same order as the activity in assays with whole blood or recalcified plasma.

We studied different heparins (standard ox heparin,  $\beta$ -heparin and heparin monosulphuric acid from ox lung, heparin from a

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dog mast cell tumour) for their antithrombin effect, anticoagulant effect on whole blood, and on plasma in one-stage prothrombin and recalcification systems. The substances were assayed on bovine, human and dog plasmas.

### Materials.

#### *Heparins.*

1. Ox heparin. The standard preparation of VITRUM, Stockholm, which contains 93 international units of heparin per mg of air-dried substance (moisture 13.1 %).

2. Heparin monosulphuric acid. Two preparations were used.

a) A sample of easily soluble barium salt obtained as a by-product in the commercial extraction of heparin from ox lung. This preparation was shown by JORPES and GARDELL (1948) to consist of roughly two parts of heparin monosulphuric acid, one part of chondroitin sulphuric acid and to contain no heparin trisulphuric acid. The barium salt was converted to the sodium salt with the use of Dowex 50.

b) Purified heparin monosulphuric acid from ox lung prepared by JORPES and GARDELL (1948) (one and the same sample as was used by YAMASHINA 1954).

3.  $\beta$ -heparin. A sample of  $\beta$ -heparin from ox lung, which Drs. WINTERSTEIN and MARBET, Hoffmann-la Roche, Basel, had courteously given to professor JORPES, who kindly placed it at our disposal.

4. Heparin from dog mast cell tumour. From a mast cell tumour of dog, kindly supplied by professor RUBARTH, Royal Veterinary College, Stockholm, heparin was obtained as a purified barium salt by the method described by MAGNUSSON and LARSSON (1955). Heparin solutions were prepared with 0.9 % sodium chloride.

#### *Plasmas.*

Human plasma, bovine plasma and dog plasma were used as clotting substrates. Citrated plasma was prepared by mixing one volume of 3.8 % sodium citrate with nine volumes of blood, and then centrifuging at 1,150 g, for 20 minutes at 4° C. The plasma samples were used for the clotting tests 2-6 hours after collecting the blood.

#### *Thromboplastin.*

Human brain thromboplastin prepared according to the method described by OWREN (1947).

#### *Thrombin.*

Thrombin Roche (Topostasin) was used.

**Table**

*Anticoagulant activity of dog mast cell heparin, heparin monosulphuric*

Heparin preparation	Human plasma					
	Inhibitory effect on the prothrombin time and recalcification time			Antithrombin effect		
	A	B	C	A	B	C
Dog mast cell tumour heparin .....	88	80	95	120		
Heparin monosulphuric acid .....	4			0.4	0.4	0.2
(cf. Methods Prep. 2 b)					—1	
Heparin monosulphuric acid .....	5—6	2	6	0.5	0.5	0.2
(Prep. 2 a)					—1	
$\beta$ -Heparin (ox lung) .....	5	10	5	4	5	3

The figures represent the activity in units of standard heparin per mg dry tition curves (cf. Fig. 1).

### Methods.

The clotting tests were performed in the manner described by NILSSON and WENCKERT (1954).

The anticoagulant activity of the various heparin preparations was studied in the following manner.

1. *The whole blood method of JALLING, JORPES and LINDÉN (1946).*
2. *The antithrombin effect.* To aliquots of 0.2 ml of citrated plasma was added 0.2 ml of heparin solution in increasing concentration. These mixtures were then allowed to stand 3 minutes in a water bath at 37° C after which 0.2 ml of a thrombin solution was added. Unless otherwise stated, the solution contained 7.5 N. I. H. units of thrombin per ml. In some systems a larger amount of plasma was used, namely 0.4 ml of citrated plasma + 0.1 ml of heparin solution + 0.1 ml of a thrombin solution containing 15 N. I. H. units per ml.
3. *The inhibitory effect on one-stage prothrombin time and recalcification time of plasma.* To 0.2 ml of citrated plasma were added 0.2 ml of thromboplastin and 0.2 ml of heparin solution in increasing concentration. After incubation at 37° C for 3 minutes, 0.2 ml of 30 mM calcium chloride solution was added and the clotting time recorded. The inhibitory effect on the recalcification time of plasma was determined with the use of the same system but without any addition of thromboplastin. These heparin titrations were done with human, bovine

**Table I.**  
**acid and  $\beta$ -heparin in tests on human, bovine and dog plasma.**

Bovine plasma						Dog plasma						Whole ox blood
Inhibitory effect on the prothrombin time and recalcification time			Antithrombin effect			Inhibitory effect on the prothrombin time and recalcification time			Antithrombin effect			The method of JALLING, JORPES and LINDÉN
A	B	C	A	B	C	A	B	C	A	B	C	
82			87			100	100	$\geq 200$	115	100	130	90
4-6			-100			-133			0.6	1	< 0.5	4
			4			1.5						
						-3						
4.5	10	3	15	20	10							8

weight. Values A, B, and C refer to corresponding parts of the heparin titra-

and dog plasmas as test bases. All plasma samples were simultaneously titrated with the heparin standard of Vitrum and the preparation to be tested.

### Results.

The anticoagulant properties of the preparations were compared with ox heparin in diagrams in which the clotting times were plotted against heparin concentrations. We found the activity of dog heparin, heparin monosulphuric acid and  $\beta$ -heparin in relation to standard ox heparin to vary with the test system, test base and heparin concentration used in the assay. Thus we could not express the activity simply in units of standard heparin.

The figures obtained are summarized in Table I. Values A, B, C refer to corresponding parts of the heparin titration curves.

Fig. 1 shows titration with dog mast cell heparin and ox heparin on human plasma in the recalcification system and the thrombin test system. Figs. 2 and 3 give corresponding titrations on bovine and dog plasma. The anticoagulant activity of the dog mast cell heparin on whole ox blood was found to be 90 units per mg dry weight. The antithrombin effect of the dog mast

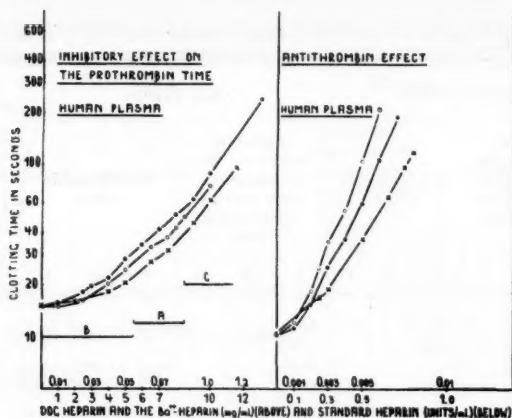


Fig. 1. The anticoagulant effect of dog mast cell tumour heparin on human plasma.

- — ○ Dog mast cell tumour heparin  
 × — × Standard heparin converted to Ba<sup>++</sup>-salt  
 ● — ● Standard heparin

cell heparin on human plasma proved higher than the inhibitory effect in the recalcification system. (Table I.) Even when compared with a barium salt of ox heparin (containing 80 units per mg by the whole blood method), the same tendency was observed. On bovine plasma the anticoagulant units in both test systems corresponded approximately to those obtained on whole ox blood. In the one-stage system with dog plasma, the shape of the heparin titration curve for the dog mast cell heparin differed considerably from that found for ox heparin. The higher the concentration of heparin, the higher the relative activity. It apparently increased from 100 units per mg at low concentrations to more than 200 at high concentrations. Similar titrations with twice the amount of plasma in the system gave the same result. This difference between dog mast cell heparin and the standard heparin could not be demonstrated in the thrombin test on dog plasma. Here the activity curve for dog heparin was essentially of the same shape as that for standard heparin.

Figs. 4 and 5 compare heparin monosulphuric acid with the standard heparin. Assayed on whole ox blood the heparin monosulphuric acid had an activity of 4 units per mg; on human

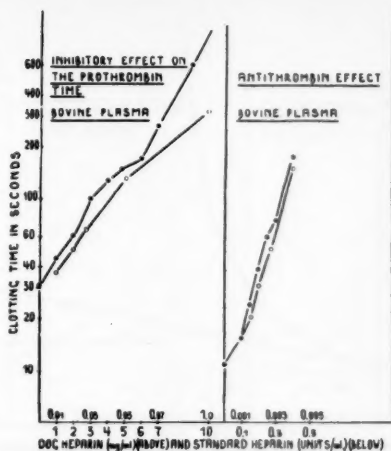


Fig. 2. The anticoagulant effect of dog mast cell tumour heparin on bovine plasma.

- — ○ Dog mast cell tumour heparin  
● — ● Standard heparin

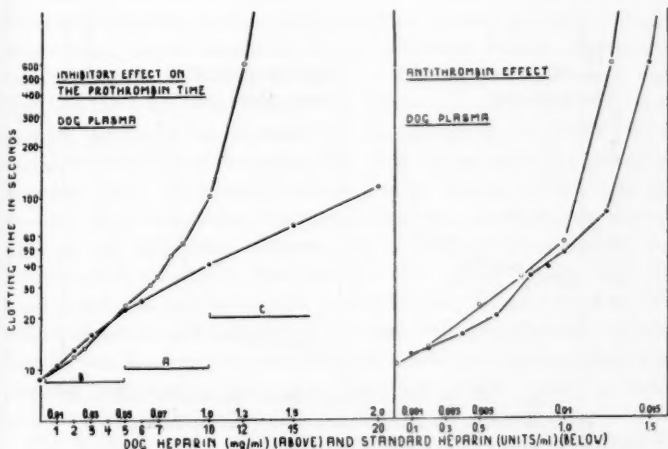


Fig. 3. The anticoagulant effect of dog mast cell tumour heparin on dog plasma.

- — ○ Dog mast cell tumour heparin  
● — ● Standard heparin

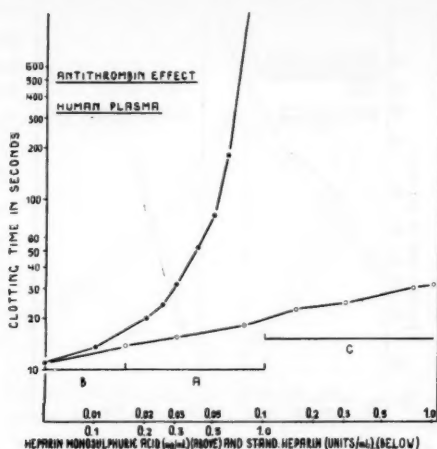


Fig. 4. The antithrombin effect of heparin monosulphuric acid on human plasma.

- — ○ Heparin monosulphuric acid  
● — ● Standard heparin

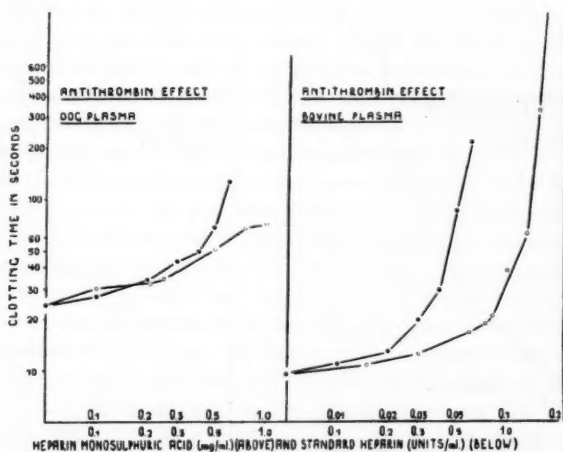


Fig. 5. The antithrombin effect of heparin monosulphuric acid on dog plasma and bovine plasma.

- — ○ Heparin monosulphuric acid  
● — ● Standard heparin

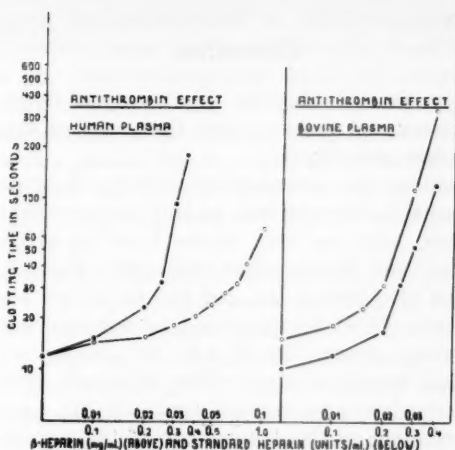


Fig. 6. The antithrombin effect of  $\beta$ -heparin on human plasma and bovine plasma.

○ — ○  $\beta$ -heparin  
● — ● Standard heparin

plasma the inhibitory effect on both the prothrombin time and the recalcification time corresponded to 4 units per mg. On the other hand, in the thrombin test system with human plasma the monosulphuric acid had an activity of only 0.4 units per mg. The use of twice the amount of plasma in this thrombin test system produced no increase in the antithrombin effect. With a smaller amount of thrombin (2 N. I. H. units per ml of thrombin solution) the antithrombin effect was as low as in the original system. However, when human plasma was replaced by bovine plasma this difference between the inhibitory effect on the recalcification time and the antithrombin effect of the heparin monosulphuric acid was not demonstrable. In dog plasma the monosulphuric acid behaved in the same way as in human plasma. The curves for heparin monosulphuric acid are derived from the purified preparation (2 b). The figures in Table I refer to both preparations (2 a and b).

plasma Fig. 6 shows that the higher effect of  $\beta$ -heparin in thrombin systems than in assays with whole blood was demonstrable when bovine plasma was used as clotting substrate in the thrombin test but not when human plasma was used.

### Discussion.

It is known that the heparin determinations differ with the method of assay and that heparins from various species differ in anticoagulant activity.

Our values for the anticoagulant activity of heparin monosulphuric acid and  $\beta$ -heparin from ox lung corresponded to those of earlier reports, when we used bovine blood or plasma for the assay (MARBET and WINTERSTEIN 1951, 1952, YAMASHINA 1954). But when we used human and dog plasma as test bases we got different results. Thus,  $\beta$ -heparin had no increased antithrombin effect on human plasma (Fig. 6, left). In addition we regularly observed, that the antithrombin effect of heparin monosulphuric acid on human plasma was markedly reduced (Fig. 4 and Table I), only corresponding to about one tenth of the activity obtained in the other assay systems.

As the only known variable in these thrombin tests was the plasma substrate, the varying antithrombin effect can presumably be ascribed to qualitative and/or quantitative differences between the heparin co-factor in human, bovine and dog plasmas. Increasing the amount of human plasma in the test system did not increase the antithrombin effect of heparin monosulphuric acid or  $\beta$ -heparin. This suggests a difference not only in concentration, but also in properties of heparin co-factor in the plasmas compared. The finding that dog mast cell heparin had a higher antithrombin effect on human plasma than on bovine plasma (Figs. 1, right and 2, right) also points to a difference in the properties of heparin co-factor.

The anticoagulant activity of the dog mast cell heparin varied markedly in the different assay systems. JAKES, WATERS and CHARLES (1942) prepared dog heparin from liver which had an anticoagulant activity of 250 units per mg, when tested with the cat whole blood method. Assayed on bovine plasma and blood our dog mast cell heparin had an activity of only 90 units per mg (Fig. 2 and Table I). Assayed on human plasma it had a higher activity in the thrombin test system (Fig. 1), and on dog plasma its activity in the recalcification system (Fig. 3, left) corresponded to between 100 and more than 200 units per mg depending on the heparin concentration employed. MARBET and WINTERSTEIN (1952) pointed out that dog heparin had the same activity

whether tested for antithrombin or antithromboplastin effect. They used bovine plasma for the antithrombin assay and human plasma for the antithromboplastin assay. In accordance with these findings of MARBET and WINTERSTEIN our dog mast cell heparin showed almost the same activity in the antithrombin assay on bovine plasma (Fig. 2, right) as in the one-stage assay on human plasma (Fig. 1, left). This was not the case, however, when we applied the dog mast cell heparin to other test bases (Figs. 1, 2 and 3).

The anticoagulant activity of different heparins in relation to the standard ox heparin thus markedly depends on the assay system which has been repeatedly stated by former authors, *e. g.* BLOMBÄCK, BLOMBÄCK, CORNELIUSSON and JORPES (1953). Judging by the present study, it is equally essential which kind of plasma is used as the clotting substrate and source of heparin co-factor. In the same way as we know that heparins from various sources differ in respect of their anticoagulant properties, it may be worth while considering possible differences also in the properties of the heparin co-factors of different plasmas.

### Summary.

Standard ox heparin, dog mast cell tumour heparin, heparin monosulphuric acid and  $\beta$ -heparin from ox lung were compared with respect to anticoagulant effect on human, bovine and dog plasma in one-stage prothrombin, recalcification and thrombin systems, and on whole ox blood. Different values for the anticoagulant activity of a certain heparin was observed when using the various assay methods, which is in conformity with the results of previous authors. In addition it was demonstrated that one and the same assay method for a certain heparin may give different results depending on the species of plasma used as clotting substrate.

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## The Renal Excretion of Urea.

By

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In 1951 FÖLDI and SZABÓ published the results of some studies based upon a couple of similar works by Russian authors, who had shown that urea is excreted not only by glomerular filtration, but also by tubular secretion. One might agree with FÖLDI and SZABÓ that a tubular secretion of urea seems a reasonable thing, especially when viewed phylogenetically. But it is a fact that urea loading has never shown a tubular secretion according to the literature of Western Europe and America.

FÖLDI and SZABÓ undertook a great many clearance studies on dogs with and without urea loads. They studied the effects of simultaneous loads of PAH and glucose, the influence of quinine, of dextroglucosyl (Diamox), caronamide and of phloridzine on the urea clearance and the clearance ratios under urea loads as well as the effect of uranyl poisoning on the urea excretion under urea load. Their results of 35 experiments were recorded in ten tables. The clearances studied were that of urea, endogenous creatinine, PAH and sometimes inulin. Their normal clearance values (without urea load) and ratios seem identical with ours. On the whole their figures give an adequate foundation for their conclusions. Without going into detail concerning the authors' discussion of their results, the main conclusions will be reported in a somewhat concentrated form, which we feel certain does not alter the meaning:

1. On applying a urea load, tubular secretion of urea takes place. (Not in all instances, but mostly.)
2. In some instances, simultaneous loads of PAH and urea diminish to a small extent the tubular secretion of urea.
3. Similarly, the tubular secretion of urea can be diminished by glucose loads.
4. As urea loads do not diminish the secretion of PAH, and secretion of urea can not be inhibited by caronamide, it seems that two separate mechanisms exist inducing the secretion of urea and PAH independently.
5. Phloridzine inhibits to a slight extent the tubular secretion of urea. This supports the assumption that the reabsorption of glucose and secretion of urea on one hand, and the secretion of PAH and urea on the other, are not competitive processes sharing a common source of energy.
6. Quinine, which inhibits the renal formation of ammonia and the action of glutaminase, does not exert any influence on the secretion of urea.
7. At normal plasma urea levels tubular secretion of urea is masked by rediffusion processes dominating in such conditions.
8. Carbon anhydrase inhibitor (deseptyl) completely arrests the tubular secretion of urea.
9. Uranyl acetate poisoning of the renal tubules inhibits the tubular urea secretion.
10. This latter fact as well as the enhancement of rediffusion by way of the impaired tubular epithelium, provides an explanation why the tubular urea secretion can not be detected in patients suffering from uremia.

When one of us talked about this Hungarian paper to DONALD D. VAN SLYKE, he stated that he and his associates in the past had made numerous urea load studies on dogs without seeing any sign of tubular secretion. As the findings of FÖLDI and SZABÓ are in great contrast to common concepts at least among Anglo-American and Scandinavian authors, a reconsidering of the question seems to be warranted.

### Methods and Experimental Procedure.

We made use of 5 female dogs weighing between 16 and 23 kg. Eighteen experiments were done, and we tried to repeat the basic studies of FÖLDI & SZABÓ. The dogs were trained laboratory animals. No anesthesia was used. In some experiments the dogs had been put on a low protein diet beforehand in order to reduce the blood urea values. In other experiments the dogs were on their regular diet. Water was given by stomach tube from 25 to 70 minutes before zero time, in the first experiments  $2 \times 500$  ml and in the later experiments only

500 ml, as diuresis in the first experiments tended to be rather large. Urine was obtained through an indwelling catheter, venous infusions through canula in vena saphena. Blood for analysis was obtained through venous punctures. The procedure was the well known clearance technique as originally described by HOMER SMITH.

The only difference in experimental procedure that we can see is the use of different laboratory methods.

The following renal functional tests were performed: 1) Urea, 2) Creatinine and 3) Inulin clearances.

**Urea:** For the determination of urea in plasma and urine the micro-diffusion method of CONWAY (1933) was used and with some slight modifications from STEINITZ (1939) using boric acid and bromocresol-green as indicator. Urease from Sigma in phosphate buffer was used. From the total amount of ammonia in urine the preformed ammonia was subtracted. For the titration use was made of a Rehberg micro-buret using approximately 0.07 N  $H_2SO_4$ . Standard with known amount of urea was run every day.

**Inulin:** For the determination of inulin use was made of the diphenylamine procedure described by ROLF, SURTSHIN and WHITE (1949) using 2.5 g of diphenylamine. Heating temperature and heating time was the same. For the precipitation of blood protein the Somogyi solution 1/10 was used and further diluted to 1/20. Urine dilution usually was 1/100 or 1/200. A urine blank and a blood blank taken before infusion were always run at the same time. The blood was read against precipitating reagent + diphenylamine, the urine against water and diphenylamine. Inulin was read at a wavelength of 635 m $\mu$  by means of a Uvispek photoelectric colorimeter.

**Creatinine:** For the determination of total creatinine use was made of Wu's modification (1922) of the FOLIN and WU method for protein precipitation. The colour reaction was carried out with the weaker picric acid (BONSNES and TAUSKY's modification) (1945). True creatinine was determined by use of Lloyd's reagent as described by HAUGEN and BLEGEN (1953).

FÖLDI and SZABÓ state that urea was determined according to Balint's method, but otherwise they do not discuss methods, referring to a previous paper with which we are unacquainted.

## Results.

We have made 12 experiments in 5 dogs in which the Wu's modification of the FOLIN and WU method for determination of total creatinine were used. Table I shows a single representative experiment. In table II the average values in all 12 experiments of plasma urea, total plasma creatinine, urea clearance, total creatinine clearance and of ratio urea clearance/total creatinine clearance are presented. We did not by intravenous infusion of

Table I.

Period	Time	Urine Flow	Plasma		Urine		Clearance		Clearance Ratio
			Urea	Creatinine	Urea	Creatinine	Urea	Creatinine	
	min.	$\frac{\text{ml}}{\text{min.}}$	$\frac{\text{mg}}{100 \text{ ml}}$			$\frac{\text{ml}}{\text{min.}}$	$\frac{\text{ml}}{\text{min.}}$	$\frac{\text{Urea}}{\text{Creatinine}}$	
1	20	4.00	16.3	0.88	96.5	6.13	38	47	0.81
2	12	3.96	16.3		79.6	5.38	38	50	0.76
3	32	6.85	107	0.85	508	3.88	40	40	1.00
4	30	7.96	191	0.90	757	3.38	38	37	1.03
5	19	4.32	238	1.03	1,478	5.62	41	38	1.10
6	19	4.00	303	1.03	1,800	6.00	39	39	1.00
7	16	3.90	364	1.08	1,900	5.58	35	36	0.97

Experiment June 14, 1955, dog R, 16 kg, three days maintained on a low protein diet. 50 and 20 min. before zero time 500 ml + 500 ml water by stomach tube. From 42 min. after zero time to the end of experiment 10 per cent urea inf. 4 ml/min.

Table II.

Dog	Experiment	Clearance Periods	Plasma Urea Max. value	Total Plasma Creatinine	Clearance Urea	Clearance Total Cr	Clearance Ratio
			$\frac{\text{mg}}{100 \text{ ml}}$	$\frac{\text{mg}}{100 \text{ ml}}$	$\frac{\text{ml}}{\text{min.}}$	$\frac{\text{ml}}{\text{min.}}$	$\frac{\text{Urea}}{\text{Creatinine}}$
B	1	3	<sup>1</sup> 29	1.0	47	54	0.87
"	3	5	189	1.09	39	45	0.86
"	5	5	378	1.12	41	43	0.96
G	2	2	<sup>1</sup> 24	1.0	57	62	0.93
"	4	5	267	1.17	48	52	0.92
"	6	5	366	1.12	43	47	0.92
R	7	7	364	0.96	38	41	0.95
"	8	5	209	0.96	56	45	1.25
"	9	5	159	1.01	44	43	1.02
K	10	7	175	1.17	47	44	1.07
"	12	5	179	1.17	35	39	0.90
D	11	7	170	1.20	30	34	0.88
Average values in all experiments				1.08	44	46	0.96

Average values in each of 12 experiments in which the Wu's modification of the Folin and Wu's method for determination of total creatinine were used.

<sup>1</sup> No urea infusion performed in this experiment.

Table III.

Period	Time min.	Plasma			Clearance Values				Clearance Ratio		
		Urea mg 100 ml	Creatinine Total mg 100 ml	Creatinine True mg 100 ml	Urea ml min.	Total Creatinine ml min.	True Creatinine ml min.	Inulin ml min.	Urea Total Creat.	Urea True Creat.	Urea Inulin
1	29	83.8	1.0	0.80	36	40	49		0.80	0.73	
2	31	169.5	1.0	0.82	33	37	46	44	0.89	0.72	0.75
3	19	222.0	1.02	0.81	37	36	45	44	1.03	0.83	0.81
4	16	264.0	1.02	0.82	35	39	48	44	0.90	0.73	0.79
5	16	291.0	1.02	0.82	37	38	47	46	0.97	0.79	0.84

Experiment Aug. 8, 1955. Dog R (the same as in table I), three days maintained on a low protein diet. 25 min. before zero time 500 ml water by stomach tube. From zero time to end of experiment 50 ml 5 per cent inulin + 500 ml 10 per cent urea inf. 4 ml/min. Zero plasma urea: 34.6 mg/100 ml.

Table IV.

Dog	Experi- ment	Clear- ance Period	Plasma			Clearance Values				Clearance Ratio		
			Urea Max. Val.	Creat. Total	Creat. True	Urea	Creat. Total	Creat. True	Inulin	Urea Creat. Total	Urea Creat. True	Creat. True Inulin
R	13	3	24	0.98	0.81	39	46	55	56	0.85	0.70	0.98
"	15	5	290	1.10	0.81	36	38	47	45	0.94	0.76	1.05
D	14	3	155	1.13	0.68	40	42	68	61	0.95	0.59	1.11
"	16	5	315	1.15	0.85	34	44	50	51	0.77	0.69	0.98
"	18	5	303	1.16	0.85	52	57	74	80	0.91	0.70	0.93
K	17	5	270	1.18	0.78	48	44	68	61	1.08	0.71	1.11
Average values:				1.11	0.80	41	45	60	59	0.91	0.69	1.03

Average values in each of 6 experiments in which true creatinine and inulin clearance are determined.

<sup>1</sup> No urea infusion performed in this experiment.

urea succeed in raising urea clearance significantly above creatinine clearance except in one experiment (Dog R, exp. no. 8), although blood urea values were raised to the same levels as in the experiments of FÖLDI and SZABÓ or even higher. We are unable to explain the high ratio urea clearance/total creatinine clearance in this single experiment (highest value 1.39, average value 1.25 in five clearance periods) by failure in the experimental procedure.

In the further experiments we therefore made use of true creatinine clearance and inulin clearance, in order to get a control of the true creatinine. Six such experiments were done in 3 dogs. Table III shows a representative experiment. Table IV gives the average values of all 6 experiments in the same manner as shown in table II.

As will be seen, the two series of experiments give nearly the same average values for total plasma creatinine (1.08 and 1.11 mg/100 ml), urea clearance (44 and 41), total creatinine clearance (46 and 45) and hence for the ratio urea clearance/total creatinine clearance (0.96 and 0.91). This fact shows that the experimental procedure is reliable. Table IV shows the marked difference between total and true creatinine, and on the other hand the good correlation between the true creatinine clearance and the inulin clearance (average values 60 and 59).

### Discussion.

Our results are in complete disagreement with the results obtained by FÖLDI and SZABÓ, and we thus were unable to confirm their finding of tubular secretion of urea at high plasma urea concentrations. We have been unable to find a reasonable explanation for this great discrepancy, and have even been wondering whether the use of different races of dogs may be the reason, just as the Dalmatian coach hound shows peculiarities in regard to the uric acid excretion. We have however no real foundation for such speculations. It is noteworthy that although differences in chemical methods exist, this cannot explain the differences in results as both in FÖLDI and SZABÓ's and in our own experiments the clearance values before urea infusions were normal.

As we were unable to demonstrate any sign of tubular urea secretion after urea loading we did not feel it of any interest to

continue our studies along the same lines as used by FÖLDI and SZABÓ and referred to above.

We want to emphasize the importance of using what we call true creatinine determinations instead of total chromogen values in studies of renal clearance ratios in dogs, as the non creatinine chromogen values are significantly higher in dogs than in man, and on the other hand the values of true creatinine and inulin show very good correlation.

If total chromogen is used instead of true creatinine the ratio urea clearance/creatinine clearance will be found too high. This is in complete accordance with our findings in humans (HAUGEN and BLEGEN 1953). When using true creatinine the same ratio never exceeds 1.0. SHANNON (1938) has shown that the same ratio extrapolates to 1.0 during forced diuresis, indicating that there is no active reabsorption of urea. This phenomenon is also seen in our experiments.

### Summary.

Based upon works by Russian authors and upon own clearance studies the Hungarian authors FÖLDI and SZABÓ in 1951 conclude that urea loading promotes tubular secretion of urea. In an attempt at reinvestigating the problem we made 18 clearance experiments in 5 dogs with altogether 87 clearance periods. Intravenous urea loads was performed in 14 experiments with 76 clearance periods. We never succeeded in raising urea clearance significantly above creatinine clearance. It is emphasized that true creatinine which shows the same values as inulin, not total chromogen determination has to be done for obtaining correct ratios.

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## ADDENDUM.

After this paper was sent to press, a study was published in the December 1 1956 number of *Klinische Wochenschrift* by R. HEINTZ, S. GÖRLITZ and E. SCHNEIDER under the heading: "Untersuchungen über die renale Harnstoff- und Inulinausscheidung bei akutem Harnstoffanstieg in Serum." (Page 1227—1231.) Eight normal individuals were subjected to urea loading with a technique practically identical with ours. After urea load, urea clearance lay definitely above inulin clearance in 7 out of 39 clearance periods, a result that corresponds to that of FÖLDI et al. There are however several objections to be made. The increase in the ratio urea cl./inulin cl. is often due to a great and wholly inexplicable fall in the inulin clearance. The ratio creatinine cl./inulin cl. is in only two of the eight individuals within the limits usually accepted as normal, and sometimes widely above. In one of their cases the ratios of creat. cl./inulin cl. and urea cl./inulin cl. both rose to more than ten times the zero value. Further, a variability in clearance values is seen that in our experience usually is due to experimental errors.

In our opinion their figures do not validate the conclusions based upon them.

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University of Oslo, Norway.

## **The Electroencephalogram of the Codfish (*Gadus Callarias*).**

### **Spontaneous Electrical Activity and Reaction to Photic and Acoustic Stimulation.**

By

PER STOCKFLETH ENGER.

Received 12 December 1956.

#### **Introduction.**

Echo sounders (frequency range 12—30 k. cycles) for localization of fish shoals are at present a common piece of equipment on board the bigger fishing boats. Some fishermen, however, believe that the use of echo sounders frightens the fishes. This would mean that fishes were able to perceive the high-frequency mechanical vibrations emitted from the echo sounders. The present investigation was started in an attempt to make out whether or not this assumption was true. A method which might give an answer to this question is the arousal reaction in the electroencephalogram (EEG). This well known reaction consists in a "desynchronization" of the relatively slow waves characteristic of a resting EEG, and may be produced by sensory stimuli of both exteroceptive (visual, acoustic, olfactory, tactile, nociceptive) and interoceptive (proprioceptive, visceral) origin. According to RHEINBERGER and JASPER (1937), the arousal reaction in the human EEG is only obtained when such stimuli arouse the subject to alertness and attention. It seems likely, therefore, that the same changes might take place in the EEG of fishes if they were able to perceive echo sounding.

EEGs have previously been obtained from a number of mammals, and also from frog (GERARD and YOUNG 1937, LIBET and GERARD 1939, MÜLLER 1951) and salamanders (PETERS and VONDERAHE 1954). The EEG varies somewhat from species to species, but is fundamentally the same in man and all the animals so far investigated. In fishes, records have been obtained from isolated brains only. It was therefore deemed necessary, in a preliminary investigation, (1) to develop a method for EEG-recording in the conscious (*i. e.* unanaesthetised) fish and (2) to study the spontaneous EEG of fishes at rest, and its reaction to various types of sensory stimulation, such as light and sound. The present paper deals with these problems. EEGs have also been recorded in anaesthetised fishes.

### Material and Method.

*Material.* The present results are based on an investigation of 16 codfishes. The body weight ranged from 0.3–2 kg. In addition 16 preliminary experiments were performed, in order to obtain a useful method, especially for trying out different types of electrodes.

*The type of electrode* finally chosen (fig. 1 B) was a silver-chlorided silver wire, 1.5 mm thick, bent at a right angle and insulated except at the tip. The electrode was connected to a thin flexible, insulated wire.

*The electrode implantation* was done under urethane anaesthesia (1 ml 25 per cent urethane per kg body weight given intraperitoneally). The fish was lying on a table with a stream of sea-water passing through the gills. The skin was kept moistened by means of wet filter paper. The size of the brain in relation to the head is seen in fig. 1 A. The neurocranium was exposed, and six electrodes — three on each side — were implanted through six small burr holes in the skull. The chlorided tips of the electrodes were in contact with the surface of the brain tissue (fig. 1 B). The electrodes were fixed to the cranium by means of a quick-drying acryle compound which also kept the neurocranium waterproof. The electrode wires were led to a caudal opening in the skin (fig. 2).

The following bipolar *leads* were used (fig. 1 C):

- I Between right and left telencephalon.
- II Between right and left mesencephalon.
- III Between right and left medulla oblongata.
- IV Between left tel- and mesencephalon.
- V Between left mesencephalon and medulla.
- VI Between right tel- and mesencephalon.
- VII Between right mesencephalon and medulla.

After the implantation of the electrodes the fishes were kept in sea-water (10–12° C) for 18–48 hours before the EEG-recording. The

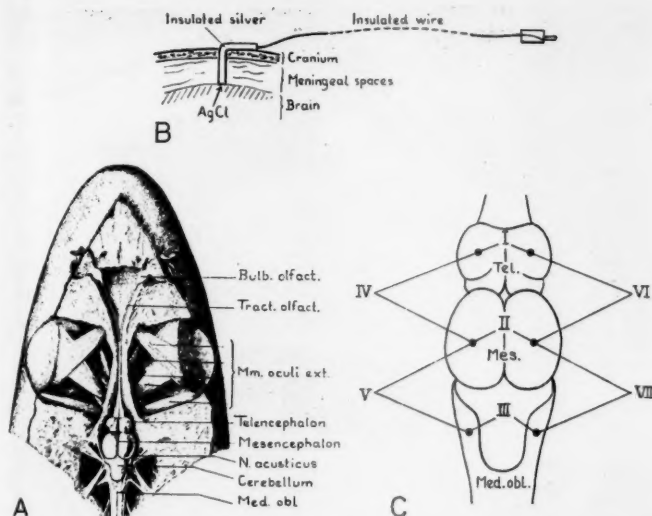


Fig. 1. A. Dorsal view of head and brain of cod (after MÜLLER 1922, fig. 103). B. Schematic representation of type of electrodes used. C. Position of electrodes. The roman figures I—VII show the leads used.

fishes seemed to recover from the operation after about three hours. During the recording, the fishes were placed in a 50 litre metal container filled with sea-water saturated with oxygen ( $8-12^{\circ}\text{C}$ ).

An eight-channel Ediswan electroencephalograph (type MK II) was employed. The upper frequency filter commonly used was 75 c/sec., and a time constant of 0.03 sec. This relatively short time constant was necessary in order to reduce the artefacts caused by the respiratory movements of the gills (0.3—0.8 c/sec.), but these were still present even with this short time constant (figs. 3 and 4). However, in control experiments a time constant of 0.3 sec. was employed and the frequency filters were switched off.

**Photic and acoustic stimulation.** Photic stimulation was carried out by means of a pocket electric torch, or a stroboscope, or simply by switching on and off the electric lights in the room. Precautions were taken to prevent any simultaneous acoustic stimulation. The stroboscope had a frequency range of 1—1000 flashes per sec. and could be connected to a loudspeaker serving as an audio-stimulator. The loudspeaker was placed about one meter from the container. It made no difference if the loudspeaker was in direct contact with the metal.

**EEG under anaesthesia.** The fishes were killed by a lethal dose of barbiturate (0.7 ml Nembutal per kg bodyweight). In some fishes the EEG was obtained at the different depths of anaesthesia.

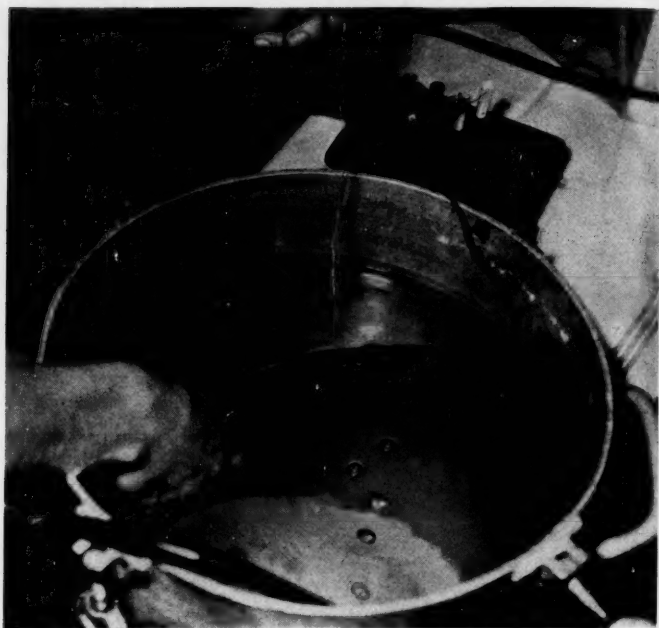


Fig. 2. The fish in the container with the electrode wires in position. The water was oxygenated through a rubber tube.

Finally, the position of the electrodes on the surface of the brain was controlled after exposure. Seven of the brains were also fixed and paraffin sections made for microscopical identification of the electrode sites. However, the macroscopical identification was the most important in this work because the electrodes were placed on the brain surface.

## Results.

### A. "Spontaneous" EEG in Cod.

#### (a) Unanaesthetised fishes.

The "spontaneous" electrical activity of the brain, *i. e.* the activity recorded in the EEG when the fish was at rest, varied considerably in different fishes as well as in the same fish at different times during the recording, which lasted from 3 to 5 hours.

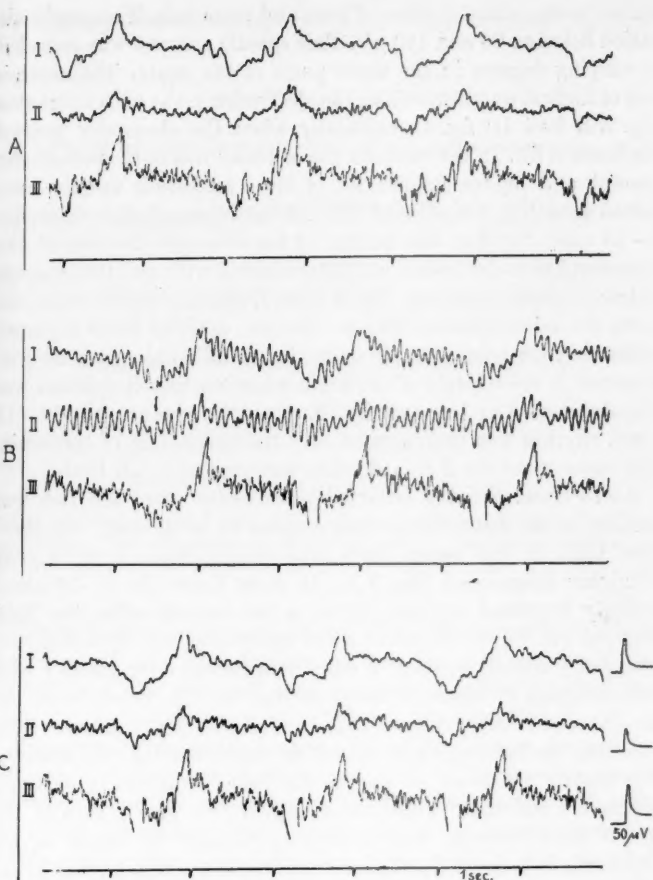


Fig. 3. The "spontaneous" EEG in non-anaesthetized cod. A. Light and quiet in the container and room. B. After 3 sec. darkness, still quiet. C. After 3 min. darkness, still quiet.

*Frequency range 8–13 c/sec.* The most dominant rhythm was a frequency of 8–13 c/sec. (fig. 3 B leads I–II; fig. 4) which was seen in all fishes for periods of varying length. The frequency usually ranged from 10 to 12 c/sec. and was, in each fish, fairly constant, with variations of about 1–2 c/sec. only. This activity usually appeared as spindles of 0.5–5 sec. duration and was very

similar to the alpha-rhythm of man and mammals. The amplitude varied between 20 and 160  $\mu$ V. This spindle activity was recorded in varying degrees in the three parts of the brain. The rhythm was of highest amplitude from the electrodes in the mesencephalon (fig. 3 B lead II; fig. 4), especially when the electrodes pressed the brain a bit. In the medulla this activity was only sporadically present and higher frequencies of lower amplitude usually dominated here (fig. 3 A—C lead III). At the telencephalon-electrodes 8—13 c/sec. rhythm was usually of lower amplitude than at the mesencephalon-electrodes, and intermingled with greater amounts of low-frequency activity. Fig. 4 (lead I) shows a typical recording from the telencephalon. The 8—13 c/sec. activity never occurred isolated in the telencephalon or in the medulla oblongata. It only occurred in these parts of the brain when the mesencephalon was also dominated by this activity. Further, the appearance of 8—13 c/sec. rhythm was synchronous over the two halves of the brain. The same was true if this rhythm was present in all leads.

8—13 c/sec. activity occurred most easily when the fish was resting in the dark. If the fish seemed to be at rest, but there was light in the room, there was usually some intermingling of higher frequencies (fig. 3 A). In some fishes the 8—13 c/sec. activity appeared immediately or a few seconds after the light was put off. In the course of a few minutes or less, however, this changed gradually again to a more irregular activity, usually with intermingling of higher frequencies (fig. 3 C). In other fishes the 8—13 c/sec. activity did not appear until a couple of minutes from the time the light was put off, but in these cases the spindle activity usually persisted as long as the fishes remained in darkness (longest consecutive experiment approx. 10 min.). Finally the 8—13 c/sec. activity was sometimes difficult to obtain at all, especially in unsettled fishes or when there was much noise in the room.

*Frequency range 14 — approx. 22 c/sec.* This activity was usually of lower amplitude than the 8—13 c/sec. activity and apt to be irregular so that the frequency was often difficult to count. In tracings with fairly regular high frequencies, up to 32 c/sec. were counted, which corresponds approximately to the highest frequencies recorded in the human EEG.

The 14—32 c/sec. activity predominated in the medulla oblongata (fig. 3 A lead III; fig. 4 lead III) and it was constant there in all fishes whether in light or darkness, regardless of which rhythm

Fig.  
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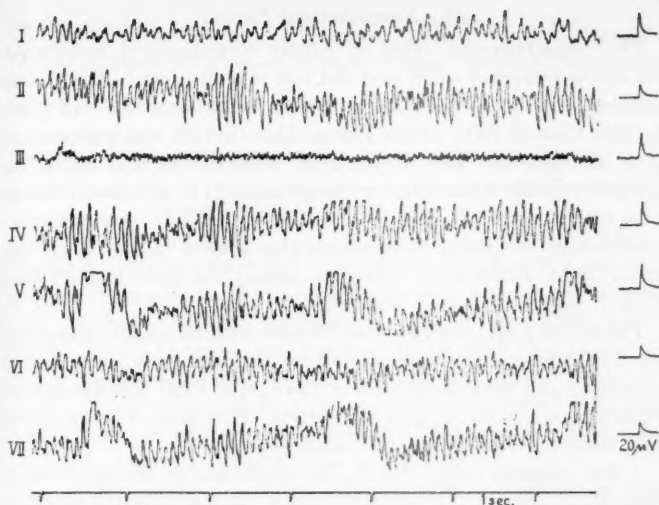


Fig. 4. The "spontaneous" EEG in non-anaesthetised cod in the dark. Notice the synchronous bilateral appearance of spindle activity. Respiration artefacts are clearly seen in leads V and VII.

dominated the rostral parts of the brain. The amplitude varied from 5 to 15  $\mu$ V. High frequencies were, as mentioned, also recorded from the tel- and mesencephalon, especially when the room was light or noisy or when the fish was unsettled.

*Frequency range under 7 c/sec.* While the highest frequencies were most pronounced in the caudal part of the brain (medulla oblongata), and the intermediate (8—13 c/sec.) in the mid part, the lowest frequencies were commonest in the rostral part, the telencephalon (fig. 4, lead I). 4—6 c/sec. activity was recorded fairly often; 2—3 c/sec., the lowest frequency observed, was less common. The amplitude was generally slightly lower than the maximal amplitudes in the 8—13 c/sec. spindles. The slow activity in the telencephalon persisted whether the room was light or dark, and was, on the whole, more stable than the 8—13 c/sec. activity. In the mesencephalon, where the low frequencies were usually less pronounced than in the telencephalon, the slow rhythms appeared clearly when 8—13 c/sec. activity was not present, and seemed to appear synchronously in the two anterior brain areas and over both hemispheres.

## (b) Anaesthetised fishes.

The "spontaneous" EEG in lightly anaesthetised cod (when the fishes remained quiet and did not react to touch, but did to cutting), showed a much more marked dominance of 8—13 c/sec. rhythm than in fully awake fishes. This rhythm was pronounced both in light and dark under light anaesthesia, and showed a marked spindle form. Higher frequencies (14—32 c/sec.) which were often present in the "spontaneous" EEG in non-anaesthetised fish in the light, were practically absent in the same fish under light anaesthesia, while the amount of slow activity (less than 7 c/sec.) increased somewhat.

The 8—13 c/sec. activity was reduced with increasing anaesthesia, and, under deep narcosis (where the fish no longer reacted to cutting and usually lay upside down), the EEG was dominated by low frequencies of high amplitude. This slow wave activity was most pronounced in the telencephalon.

A few minutes before death, the amplitude of the slow waves diminished, the tracing became "flatter" and all activity vanished 1—2 min. after the respiratory movements stopped.

**B. EEG on Photic and Acoustic Stimulation.**

## (a) Arousal reaction.

*Photic stimulation.* The arousal reaction usually appeared in the cod on all three kinds of "continuous" photic stimulation (cf. methods). The reaction was more clearly seen when the EEG showed a distinct 8—13 c/sec. spindle activity before the photic stimulation. Fig. 5 shows a typical example. It can be seen that the spindle activity, which, in the dark, predominates in the leads from the tel- and mesencephalon, is replaced by faster rhythms — primarily of frequency 18—32 c/sec. of relatively low amplitude. In the medulla oblongata where the high frequencies usually predominated, the arousal reaction was less clear or did not appear at all.

The high frequency activity (18—32 c/sec.), which replaced the 8—13 c/sec. rhythm in the arousal reaction, was usually most obvious in the mesencephalon, probably because the latter rhythm predominated here.

The amplitude of the high frequencies increased slightly with the intensity of the light, and in some fishes, with strong light,

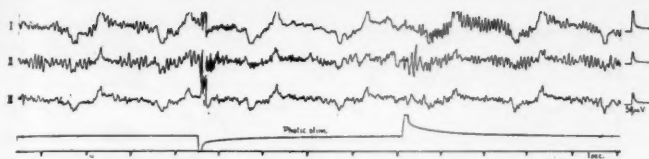


Fig. 5. Blocking of 8—13 c/sec. activity in non-anaesthetised cod by sudden illumination (stroboscope, 800 flashes/sec.).

the amplitude even exceeded that of the 8—13 c/sec. activity (fig. 6). The amplitude of the high frequencies was usually largest at the beginning of each stimulation, and declined after a few seconds (fig. 5). In some cases though, the induced high frequency activity persisted as long as the stimulus was on, for instance for periods up to a couple of minutes. Simultaneous with the reduction of the amplitude, there was in all fishes an intermingling of lower frequencies (14—18 c/sec.).

Photic stimulation caused no demonstrable alteration in the slow wave activity (less than 7 c/sec.).

When the photic stimulation was interrupted, the 8—13 c/sec. activity re-appeared either at once (fig. 5) or more gradually in 1—2 min. In the first case, the spindle activity had a slightly higher frequency (1—2 c/sec.) immediately after the interruption, as compared to the pre-stimulatory value. After a very few seconds, however, the frequency reverted to its previous level. This interesting phenomenon has also been observed in man (JASPER 1936, FARBRÖT 1954).

Photic stimulation of lightly anaesthetised fishes caused alterations in the EEG similar to those seen in the awake fishes, but here the 8—13 c/sec. rhythm was replaced by 14—18 c/sec.

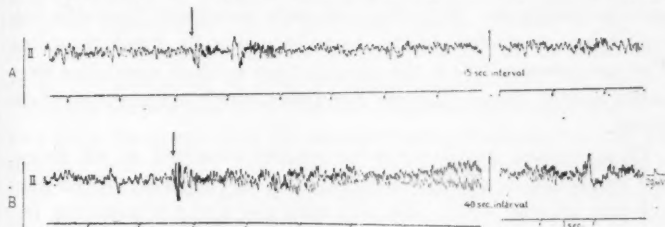


Fig. 6. Effect of different intensities of light on the EEG in non-anaesthetised cod. (A) Weak lighting by a pocket torch. (B) Stronger lighting from a roof light. The arrows indicate when the light was put on.

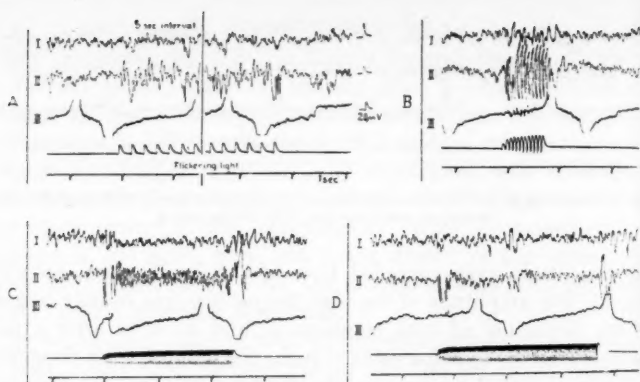


Fig. 7. EEG in non-anaesthetised cod on photic stimulation of frequencies of (A) 6, (B) 15, (C) 33, and (D) 48 flashes per sec. respectively. In this fish, isolated potentials were recorded with a stimulation frequency of up to 40 per sec., but not clearly over this frequency (D).

activity. On more prolonged photic stimulation under light anaesthesia, the 8–13 c/sec. rhythm returned after 1–2 minutes in spite of continuous stimulation.

*Acoustic stimulation.* Acoustic stimulation did not cause an arousal reaction similar to that produced by photic stimulation. As mentioned earlier, the 8–13 c/sec. activity was only slightly developed if there was much noise in the room.

#### (b) Evoked potentials.

*Photic stimulation.* Evoked potentials on flicker stimulation were recorded from the mesencephalon electrodes (fig. 7) and, in some fishes also from the telencephalon, but never from the medulla oblongata. Recording of such potentials from the telencephalon electrodes might have been due to direct recording of action potentials from the optic nerves, as these electrodes were rather deeply placed in these few fishes, fairly adjacent to these nerves.

These evoked potentials were readily obtained in all fishes. The amplitude increased somewhat with the intensity of the light and was greatest (100–170  $\mu$ V) with the lower stimulating frequencies. In some fishes, the amplitude was maximal with a stimulating frequency of 1–15 c/sec., and in other with a frequency of 10–20 c/sec. (fig. 7 B). The amplitude decreased on

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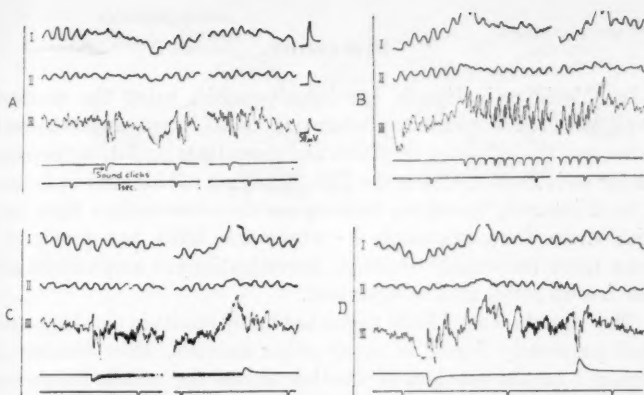


Fig. 8. EEG in non-anaesthetised cod during acoustic stimulation of frequencies (A) approx.  $1\frac{1}{2}$ , (B) 11, (C) 70 and (D) 175 clicks per sec. Tracings A, B, and C only show the start and finish of the stimulation; there was 2—3 secs. intervening interval.

increasing flicker frequency, and was only a few microvolts when the stimulating frequency reached the upper limit for recording isolated action potentials (30—40 potentials per sec.). Still higher frequencies of stimulation had the same effect on the EEG as continuous light (fig. 7 D). This upper limit sank under anaesthesia and was 10—15 c/sec. in deeply anaesthetised fishes.

*Acoustic stimulation.* Evoked potentials in response to click stimulation were only recorded in the medulla oblongata and were never seen in other parts of the brain (fig. 8). The amplitude of these potentials reached its peak (80—110  $\mu$ V) with a stimulating frequency of about 25 clicks per sec. The evoked potentials followed frequencies up to 100—140 clicks per sec. When the frequency was more than 80 clicks per sec., two or three high spike-like potentials of large amplitude followed by two to four rhythmic slow waves of 0.5—1 sec. duration were seen (fig. 8 D, lead 111). No upper limit for the stimulating frequency giving this reaction was found. The reaction was somewhat inconstant in some fishes and seemed to decrease on repeated stimulation. In one fish it was best seen during anaesthesia.

At the cessation of the acoustic stimulation, there was either no response, or a spike potential followed by high frequency waves of approx. 0.2 sec. duration.

### Discussion.

In this investigation it has been possible, using the method developed, to record the "spontaneous" EEG in non-anaesthetised fishes and the influence of photic and acoustic stimulation thereon. As far as is known, this is the first recording of this kind in fishes. It is of interest, therefore, to compare the observations from fish with those from mammals — where the EEG has previously been most thoroughly studied. Investigations in amphibians are too few to serve as a comparison.

The "spontaneous" EEG in cod has many points in common with that previously found in many other animals. Most interest is centered on the problem of whether or not the middle frequency range in cod, 8—13 c/sec. activity, corresponds to the alpha rhythm in mammals and man. There are many facts in favour of this being so. Rhythmic activity of this frequency in cod shows, as in all mammals so far investigated, a more or less clear spindle form. Further, it seems that these spindles, in cod as in mammals, are easily influenced by the surrounding circumstances, as they appear in quietness and darkness, and disappear easily if there is noise or light. The spindle activity in both fish and mammals is more pronounced under light barbiturate anaesthesia than in the fully conscious state.

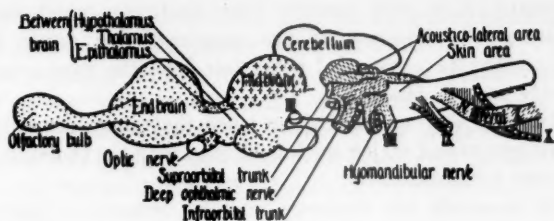
It therefore seems justifiable to conclude that the 8—13 c/sec. activity in the cod corresponds to alpha rhythm in mammals and man. It is also reasonable to suppose that this activity is connected with homologous brain structures in mammals and cod, assuming that structure and function are intimately related.

The alpha rhythm in mammals is most pronounced in the following places: the thalamic reticular system (midline and intralaminar nuclei) (MORISON, FINLEY and LOTHROP 1943, STARZL and MAGOUN 1951), caudate nucleus, and, in certain regions of the cerebral cortex, *viz.* the association zones (STARZL and MAGOUN 1951). These observations have been obtained from experiments in the cat.

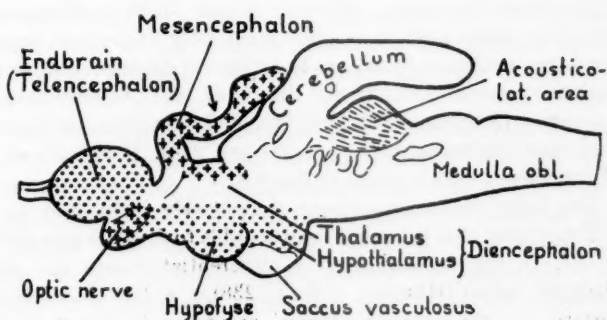
In cod, the 8—13 c/sec. activity was recorded most clearly from the mesencephalon electrodes, which were placed on the optic lobes. The mesencephalon in fish is a relatively large part of the brain surrounding the mesencephalic ventricle, as shown in fig. 9 B, which illustrates a sagittal section of the brain of one

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Fig. 9. A. Brain of the dogfish (*Squalus acanthias*) (After Herrick 1928). B. Sagittal section of cod brain. The arrow indicates the position of one of the mesencephalon electrodes. The optic lobe has been pressed down. Homologous areas are marked in the same way in A and B.

of the cods in this investigation. The nervous substance in the mesencephalon is relatively thin, in the rostral part only 1—1.5 mm thick. Post-mortem investigations of the position of the electrodes showed that the optic lobes were often somewhat pressed down (see fig. 9 B), and it was in these fishes that the 8—13 c/sec. activity was most pronounced. The distance between the tip of the electrode (when this were deeply placed) and the dorsal part of the diencephalon was a maximum of only 2 mm. Since potential changes are recordable from electrodes placed at some distance from the tissue (e. g. extra-cranial EEG recording

in man), it is very possible that electrodes which have been histologically localised to the mesencephalon (optic lobes) in cod, might have recorded potentials from the more ventral, and partly rostral, thalamus.

Is there any *anatomical* evidence that there are cells in the thalamus of cod which are homologous with the thalamic reticular system in mammals?

In *mammals* the thalamic reticular system is chiefly in the medial parts of the dorsal thalamus, and the specific nuclei are located in its lateral part. HERRICK (1928, p. 179—182) has designated the specific nuclei, neo-thalamus, in order to differentiate them from the other thalamic nuclei (*i. e.* midline and intralaminar nuclei) which together form the palaeo-thalamus. The latter is found in all vertebrates lacking cerebral cortex.

In *teleosts* the dorsal thalamus is only slightly differentiated. However, many authors describe a diffusely limited cell region in the dorsal thalamus which has had different descriptions: the inner thalamic segment (CRAIGIE and BRICKNER 1927), the sub-habenular cell region (KAPPERS 1921, p. 821) and the sensitive nucleus of the thalamus (HERRICK 1948, pp. 231 and 238). HERRICK's division of the shark brain is given in fig. 9 A.

The inner thalamic segment is relatively large, and so little differentiated that it has not been possible to divide it into different nuclei. In higher animals it is differentiated into the specific thalamic nuclei (HERRICK 1948, p. 238), *i. e.* the neo-thalamus is formed. As the palaeo-thalamus, according to HERRICK (1928, p. 182), is not reduced in phylogenesis, there is reason to believe that the palaeo-thalamus in fish is either wholly or partly composed of the inner thalamic segment, and that this is homologous with the thalamic reticular system in mammals — a system which forms a capsule round the specific thalamic nuclei. In other words, there is anatomical evidence that a thalamic reticular system exists in fish.

*The high frequency activity* (14—32 c/sec.) recorded from the tel- and mesencephalon electrodes in cod was, both in form and in the circumstances under which it appeared, very similar to beta rhythm in mammals and man. A high frequency activity predominated in the EEG of the cod when it was light, noisy and when the fish was unsettled.

Continuous, high frequency activity was only recorded in the medulla oblongata in the cod. This type of activity in the same

part of the brain has also been found in the cat (GERARD, MARSHALL and SAUL 1936) and frog (GERARD and YOUNG 1937).

The low frequency activity (under 7 c/sec.) was usually more dominant in the "spontaneous" EEG of cod than is the case in man (*e. g.* GIBBS and GIBBS 1950). However, the incidence of the slow activity was in good agreement with that found in the frog (GERARD and YOUNG 1937) and salamanders (PETERS and VONDERAHE 1954).

Finally, the question of whether the low frequency potentials (0.3—0.8 c/sec.), recorded synchronously with the respiratory movements, represent movement artefacts or genuine brain potentials should be considered. ADRIAN and BUYTENDIJK (1931) have, in the isolated brain stem of gold fish, recorded potentials with the same frequency as the respiratory movements in the intact fish. These waves were only recorded when one or both electrodes were in the medulla oblongata. SHURRAGER (1936) found similar potentials in the isolated forebrain of the cat-fish.

In this investigation, potentials were recorded from all the electrodes synchronously with the movements of the gills, and the amplitude of these potentials was greatest when the electrodes were *not* in contact with nervous tissue. When the electrodes had good contact with the brain, these potentials were either not recorded or only recorded with relatively low amplitude. For this reason it is practically certain that these potentials represent movement artefacts. When recording the EEG of salamanders PETERS and VONDERAHE (1954) also found potentials which were synchronous with the respiratory movements and these authors concluded that they were artefacts. All the same, in cod the possibility cannot be overlooked that these potentials (see figs. 3 and 4) may partially correspond to the respiration potentials found by ADRIAN and BUYTENDIJK (1931) and SHURRAGER (1936). More thorough studies with special reference to this problem are needed in order to draw a firm conclusion.

*Evoked potentials* on photic and acoustic stimulation were, as would be expected, obtained from the visual region in the mesencephalon and the acoustic region in the medulla oblongata respectively.

Here, most interest is centered on the arousal reaction on such physiological stimulation. The present investigation has shown that fish like most mammals and man, react easily to photic

stimulation by the "arousal reaction" in the EEG. The fact that the arousal reaction could not be produced by acoustic stimulation is also in relatively good agreement with what is found in mammals and man. It appears that the different sensory systems are not equally effective in bringing about the "arousal reaction".

The fact that noise in the room during the experiment could sometimes prevent the appearance of 8—13 c/sec. activity in the cod, might indicate that acoustic stimulation can cause the "arousal reaction". It is, however, not certain that it was noise in itself which was the cause of the activating picture in the EEG in these cases. Also, sometimes in man there is little alpha rhythm in the EEG and high frequencies of low amplitude predominate. As already mentioned, this occurs in anxious individuals who find it difficult to relax, or if there is much disturbance round about. The possibility cannot be overlooked that there is a similar mechanism in the cod.

It is not possible, on the basis of these experiments, to foretell whether or not a possible perception of the high frequency waves from echo sounders will be associated with an "arousal reaction" in the EEG. Only further experiments using an echo sounder for stimulating can answer this question.

### Summary.

1. The electroencephalogram (EEG) has been recorded in the cod (*Gadus callarias*) both in the awake state and under narcosis. The effects on the EEG of photic and acoustic stimulation have been observed.

2. In the awake resting state, when the fish was in the dark, the dominating rhythm recorded was 8—13 c/sec. — especially from the optic lobes (fig. 3 B, fig. 4). When the fish was unsettled or in the light, the EEG was chiefly dominated by higher frequencies, 14—32 c/sec. (fig. 3 A). Slow rhythms (less than 7 c/sec.) were most pronounced in the telencephalon and were relatively unaffected by surrounding circumstances.

3. The arousal reaction, as it occurs in mammals, *i. e.* a substitution of the dominating 8—13 c/sec. activity by a faster rhythm of lower amplitude, has been recorded in the cod EEG on sudden illumination of the fish (fig. 5). During acoustic stimulation no arousal reaction was recorded.

4. Evoked potentials on photic and acoustic stimulation were recorded from the visual area of the mesencephalon and the acoustic area of the medulla oblongata respectively. On photic stimulation up to a stimulating frequency of 40 c/sec. and on acoustic stimulation up to 100—140 c/sec., the evoked potentials followed each stimulus.

5. It is concluded that the 8—13 c/sec. activity in the cod corresponds to alpha rhythm of mammals and man, and that in the dorsal thalamus of cod there are parts that are homologous, as regards function, with the midline nuclei and intralaminar system in mammals.

#### Acknowledgments.

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## **Catheterization of the Renal Arteries in Dogs and Cats.**

By

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In the course of studies of the renal removal of injected histamine from the blood (LINDELL—WESTLING 1956), it became desirable to be able to make injections selectively into one of the renal arteries of a dog or of a cat without serious disturbance of the blood supply to the kidney. It also seemed desirable to be able to make these injections without major operative procedures in the abdomen, since such procedures may considerably disturb the renal circulation (SMITH 1956).

Selective catheterization of the renal arteries in dogs without opening the abdomen has been described by TILLANDER in 1951 and RAPPAPORTE 1952. Their catheters, however, seem likely to interfere with the blood supply of the kidney, if left in the renal artery for more than a few minutes.

Recently methods of selective angiography of the renal arteries in man have been described by ÖDMAN 1956 and EDHOLM and SELDINGER 1956. ÖDMAN uses radioopaque polythene catheters and EDHOLM and SELDINGER use polythene catheters with an internal flexible metal guide. For catheterization of the renal arteries, these authors as well as ÖDMAN provide the tip of the catheter with suitable curvature.



Fig. 1. Angiography of the right renal artery in a dog weighing 12 kg.

For the catheterization of the renal arteries in dogs and cats with as little disturbance of the blood flow as possible it is necessary to use catheters with a small external diameter. Furthermore the anatomical arrangement of the renal arteries in dogs required a catheter tip of a particular shape. These requirements have been met with in the technique to be described here.

#### **Technique of Catheterization of the Renal Arteries in Dogs.**

*Apparatus.* In our experience the renal arteries in dogs will usually leave the aorta in a caudolateral direction and then bend laterally or craniolaterally as seen in fig. 1. It seemed a priori likely that the easiest way to insert a catheter into the renal artery would be to use a catheter with a slightly bent tip and to pass it down into the aorta from a

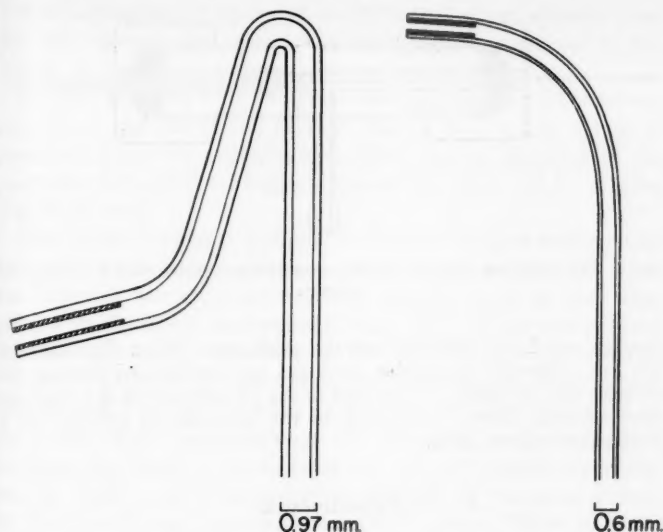


Fig. 2.

To the left the tip of a catheter intended for the renal arteries in a dog.  
To the right the tip of a catheter intended for the renal arteries in a cat.

brachial or carotid artery. However, it proved difficult to pass the catheter down into the descending aorta. And so it was decided to introduce the catheter from one of the femoral arteries. To make the catheter pass easily into the renal artery it was necessary to give the tip a form as shown in fig. 2. This form of the tip has the additional advantage of preventing the catheter from going too far out into the renal artery.

The catheter is a polythene tube, Pe 60 for bigger and Pe 50 for smaller dogs.<sup>1</sup> The tip of the catheter is provided with an internal gold tube as shown in fig. 2, to make it easily visible on fluoroscopy. During the insertion the catheter is provided with an internal guide of stainless steel, which does not extend into the bent tip. The stainless steel wire has a diameter of 0.5 mm for the Pe 60 tube and 0.3 mm for the Pe 50 tube. It is advisable not to use catheters with smaller external diameters, since they will easily slip out into a lumbar artery. To prevent clotting a slow steady infusion of saline with 0.5 mg heparin per ml is maintained by a motordriven syringe. The connection between the polythene tube and the syringe is illustrated by fig. 3. It consists of a plexiglass tube with threaded lids at each end. Below the lids are rubber packings. These as well as the lids have holes in the centre

<sup>1</sup> Made by Clay-Adams Company, INC., New York.

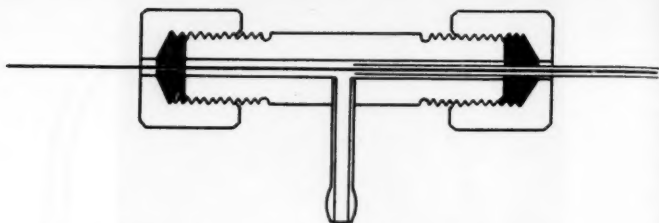


Fig. 3. The plexiglass chamber which connects the catheter with a motordriven syringe.

through which the catheter and the guide pass. When the threaded lids are tightened the rubber packings are compressed around the catheter and the guide. In the wall of the plexiglass tube is a stainless steel cannula. This is connected to the motordriven syringe via a thickwalled rubber tube.

### Experimental.

The dogs are anaesthetized with Nembutal (sodium pentobarbitone) 30 mg per kg b. wt. intravenously, and laid on their backs on a wooden tray, the legs being firmly fixed to the tray. For the localization of the kidneys a plain film of the abdomen is taken with an ordinary four valve roentgen equipment. An indicator is placed at a suitable level. The femoral artery is exposed through an incision immediately below the inguinal ligament. The artery is ligated peripherally and for the moment clamped centrally. An opening is cut in its wall, through which the catheter is inserted. The vessel is tightened around the catheter with a rubber band. Under fluoroscopical control the catheter with its guide is passed into the aorta up to the level of the indicator. The tip of the catheter is turned towards the desired side, and is moved slowly along the aorta. When it reaches the renal artery the tip suddenly moves outside the lateral border of the aorta. The guide is withdrawn under fluoroscopical control. The catheter is then firmly tied to the femoral artery. The position of the catheter can be verified by injection of 0.5—2 ml of solution of ordinary contrast medium in a 35—50 per cent solution.

### Observations.

In a group of 9 dogs it was found that the right renal artery left the aorta in a region extending from the disk between the second and third lumbar vertebrae to the middle of the second lumbar vertebra.

The corresponding region of the left renal artery extends from the disk between the second and third lumbar vertebrae to the middle of the third vertebra.

In dogs weighing 8 to 13 kg the internal diameter of the renal arteries at the site of the catheter varied from 2.2 to 4 mm as measured from the angiographies. The external diameter of the polythene tube used for bigger dogs was 1.22 mm and for smaller dogs 0.97 mm.

The injected contrast medium disappeared almost immediately from the renal arteries, and on pictures taken 2 minutes after the injection the contrast medium could be seen in the renal pelvis. On lowering the pressure with which saline was infused through the catheter blood would flow back through the catheter.

The influence of the catheter on the blood supply to the kidney will depend upon the relation between the cross section area of the lumen of the renal artery at the site of the catheter and the cross section area of the catheter. It will also depend upon the length of the part of the catheter situated in the renal artery. In the present experiments the catheter occupied from 10 to 20 per cent of the lumen of the artery. The length of the part of the catheter inserted into the renal artery was less than 1 cm.

Under these conditions the increase in resistance to the blood flow caused by the catheter is probably rather small compared to the total resistance in the blood vessels of the kidney. To estimate the influence of the catheter on renal blood flow we measured the venous outflow from the kidneys in two dogs before and after insertion of a catheter into one of the renal arteries.

**Experiments.** The dogs weighed 6 and 8 kg respectively. The anaesthesia and the position of the animals were as usual in our catheterization experiments. The animals were heparinized. Blood pressure was recorded with a mercury manometer. The blood from the renal veins was led through wide polythene tubes into the superficial jugular veins. During periods of one minute these connections were interrupted, the blood coming from each kidney was collected in measuring cylinders. The blood loss was compensated by infusion of corresponding volumes of a solution of dextrane.<sup>1</sup> After the measurements the blood was returned to the circulation. A polythene catheter was inserted into one of the renal arteries. The measurements of renal blood flow were repeated. Since no contrast medium had been injected, the position of the catheter was controlled at autopsy.

<sup>1</sup> Kindly supplied by Pharmacia A.B.

Table 1.

*Renal blood flow as measured on the venous side before and after the insertion of a catheter into the right renal artery.*

Right Kidney ml/min.	Left Kidney ml/min.
15.5	16.0
Insertion of the catheter into the right renal artery	
17.0	17.5

In neither of the experiments was there a change in renal blood flow which could be attributed to the catheter. The figures from one experiment are shown in table 1.

The effect of the catheter on the clearance of phenol red (phenol-sulfonephthalein) by the kidneys has been studied in two dogs. For details about this test of renal function see SMITH 1951.

**Experiments.** The dogs weighed 10 and 15 kg respectively. The anaesthesia and the position of the animals were as usual in our catheterization experiments. Urine was collected from polythene tubes inserted into the ureters. Blood samples were taken from a polythene tube in the femoral artery. A priming dose of phenol red, 2 mg per kg. was given by vein. It was followed by a continuous infusion of the dye. The amount infused was adjusted to balance the amount excreted in order to maintain the plasma concentration. The clearance determinations were started 30 to 60 minutes after the priming injection. The urine was collected during periods of 30 minutes. Duplicate samples of arterial blood were taken in the middle of the collection periods. The blood samples were centrifuged. After proper dilution and adjustment of pH to 10–12 the concentration of the dye in plasma and urine was determined in a Beckman spectrophotometer at a wavelength of 558 mμ where according to our experience the density was highest. Clearance determinations were made twice before and twice after the insertion of the catheter. Neither saline nor contrast medium were injected through the catheter. The position of the catheter was controlled at autopsy.

In neither of the two experiments did the catheter influence the clearance of phenol red. The results from one dog are shown in table 2.

In two dogs after renal angiography the catheter was withdrawn, the femoral artery ligated and the incision sutured. After recovery from the anaesthesia these dogs remained in good health as judged from their appetite, weight and general appearance. The

**Table 2.**

*Phenol red clearance as measured for each kidney separately before and after the insertion of a catheter into the right renal artery of a dog weighing 15 kg.*

Time min.	Phenol red excreted		Phenol red in plasma $\mu\text{g/ml}$	Clearance	
	Right $\mu\text{g/min.}$	Left $\mu\text{g/min.}$		Right $\text{ml/min.}$	Left $\text{ml/min.}$
0-30 ....	412	366	10.6	39	35
30-60 ....	429	503	12.3	35	41
Insertion of the catheter into the right renal artery					
120-150 ...	528	520	11.6	46	45
150-180 ...	504	504	11.4	44	44

angiographies were repeated after 2 weeks, and after this the animals were killed. Neither the renal arteries nor the kidneys showed any macroscopical abnormalities.

#### **Technique of Catheterization of the Renal Arteries in Cats. Apparatus and Experimental.**

The renal arteries in cats usually arise perpendicularly to the sides of the aorta. The right artery passes in a slightly cranial and the left in a slightly caudal direction. Fig. 4.

Since the renal arteries are smaller in cats than in dogs, we have used catheters with an external diameter of 0.6 mm. The available polythene tubes with this external diameter would not allow a metal guide thicker than 0.2 mm. Nylon tubes<sup>1</sup> with the same external diameter can, however, be used together with a stainless steel guide of 0.3 mm. The tip of the catheter was provided with an internal gold tube. A single bend of the tip as shown in fig. 2 will allow it to pass easily into the renal artery. This bend is straightened by means of the metal guide during the insertion of the catheter into the femoral artery and the aorta. When the tip has reached the level of the renal arteries, the guide is drawn back 1 cm. The catheter will then resume its curvature. The technique is otherwise similar to that used for dogs. Care should be taken not to pass the catheter more than 0.5 cm outside the lateral border of the aorta.

<sup>1</sup> Made by Porter Ltd., Great Britain.

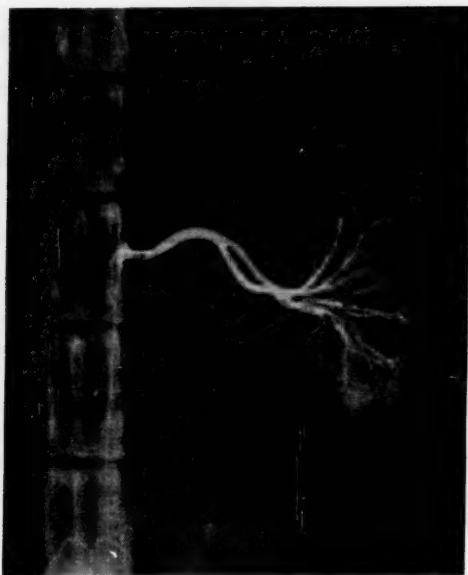


Fig. 4. Angiography of the left renal artery of a cat weighing 3.2 kg.

### Observations.

Eleven cats have been catheterized. The right renal artery left the aorta at the level of the middle of the third lumbar vertebra. The left renal artery arose a few millimeters more caudally.

In cats weighing 4–5 kg the internal diameter of the renal arteries at the site of the catheter varied between 1.7 to 1.9 mm as measured from the angiographies. The external diameter of the catheter was 0.6 mm.

Injected contrast medium disappeared from the arteries as rapidly as in dogs.

Determinations of phenol red clearances were made in two cats. The technique was similar to that used in dogs. The results from the first cat are shown in table 3. In the other cat the catheter was pushed out approximately 1.5 cm in the renal artery. After this the urine flow on the catheterized side was considerably reduced. Direct inspection revealed a spasm in the renal artery.

**Table 3.**

*Phenol red clearance as measured for each kidney separately before and after the insertion of a catheter into the right renal artery of a cat weighing 5 kg.*

Time min.	Phenol red excreted		Phenol red in plasma $\mu\text{g/ml}$	Clearance	
	Right $\mu\text{g/min.}$	Left $\mu\text{g/min.}$		Right $\text{ml/min.}$	Left $\text{ml/min.}$
0—30 ....	105	97	6.3	17	15
30—60 ....	108	107	7.1	15	15
Insertion of the catheter into the right renal artery					
120—150 ...	83	88	6.8	12	13
150—180 ...	94	95	7.8	12	12

The catheter was drawn back 1 cm. The urine flow increased again, but phenol red clearance, determined 3 hours later, showed a reduction by 30 per cent on the catheterized side.

### Summary.

A technique of catheterization of the renal arteries from a femoral artery in dogs and cats is described.

The catheter did not influence the renal blood flow as measured on the venous side.

The insertion of the catheter was not followed by any changes in the clearance of phenol red as measured for each kidney separately.

We are greatly indebted to Miss Karen Nilsson for help with the photometry and to Mr. Olle Jönsson, who prepared the gold tubes.

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From the Institute of Neurophysiology, University of Copenhagen.

## **Multielectrode Study of the Territory of a Motor Unit.**

By

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Current conceptions concerning the distribution of the muscle fibres of a motor unit are somewhat controversial and quantitative data are not available. The evenness of contraction and the absence of local contractions during weak voluntary effort have been considered to indicate that a single motor unit may be widely distributed through the muscle (DENNY-BROWN 1949, FEINDEL 1954). According to another view the fibres of a motor unit are organized in one (GORDON and HOLBOURN 1949, DENSLOW and GUTENSOHN 1950) or several small bundles (DENSLOW and HASSETT 1943) with the fibres arranged in series (COOPER 1931).

We have found that the muscle fibres pass uninterruptedly from tendon to tendon along the length of the brachial biceps (BUCHTHAL, GULD and ROSENFALCK 1955a). Therefore, a study of the distribution of the muscle fibres of a motor unit could be confined to a single cross section of the muscle. Furthermore, our previous investigation of the volume conduction of the spike of the motor unit potential indicated that whenever a spike (*i. e.* a positive-negative deflection of short duration) was recorded, active muscle fibres were situated in the immediate vicinity (BUCHTHAL, GULD and ROSENFALCK 1957). Hence, the scatter of the muscle fibres of a motor unit could be studied by recording the distribution of the spike potentials of a given motor unit over a cross section of the muscle. This study was performed using

multielectrodes containing twelve leads distributed along a length of about 25 mm. Furthermore, the parameters of the motor unit potential were related to the site of initiation, the propagation and the volume conduction of the spike potentials of the motor unit.

### Method.

*Subjects:* All experiments were performed on the brachial biceps of ten student volunteers 20–25 years of age, without neuromuscular disorder. Potentials were recorded during slight or moderate voluntary contraction at an intramuscular temperature of 36 to 37° C.

*Recording:* A three channel DISA electromyograph was used for recording. One of the multielectrode leads which picked up a potential of high amplitude was kept in constant connection with one channel of the electromyograph to observe that recording occurred from the same motor unit throughout. The spike of this potential served as time reference for the potentials recorded on the other leads. They were switched successively to the two other channels of the electromyograph; the action potential on each lead was recorded at least ten times. A 50  $\mu$ sec rectangular pulse produced a simultaneous signal on the three cathode ray tubes of the electromyograph. The properties of the amplifiers, the electrode switch and the recording technique have been described (BUCHTHAL, GULD and ROSENFALCK 1957). In some of the experiments, a double beam oscilloscope was used. It was triggered by a potential from the motor unit under investigation, picked up by a concentric electrode inserted in the innervation zone of the motor unit. Propagation of the action potential to the multielectrodes which were inserted outside the innervation zone provided sufficient delay to allow recording of the entire action potential on the oscilloscope. In this way *all* the discharges of the motor unit were recorded thereby reducing the amount of film necessary for recording and the time over which a constant voluntary effort had to be maintained by the subject.

*Multielectrodes:*<sup>1</sup> It was desirable that the multielectrode scan action potentials over the larger part of the muscle cross section. Since no more than twelve leads could be placed in a cannula one mm in external diameter, twelve 1.5 mm long leads were distributed over a length of 25 mm with a spacing of 0.5 mm (Fig. 1 B). The leads were 0.1 mm platinum wire embedded in heat hardened araldite. They were situated along that side of the cannula opposite to the oblique tip area. With the electrode inserted at right angles to the longitudinal axis of the muscle this ascertained that potentials were led off from those fibres which were least displaced by the tip of the electrode.

<sup>1</sup> The multielectrodes are manufactured by DISA Elektronik, Copenhagen.

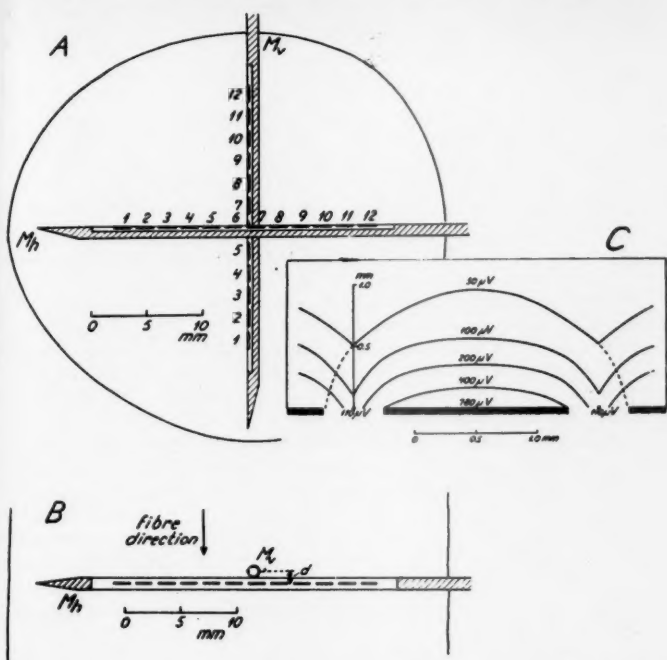


Fig. 1. Two multielectrodes ( $M_v$ ,  $M_h$ ) inserted at right angles to each other into the muscle.

A. Section transversely to the fibre direction.

B. Section longitudinally to the fibre direction;  $d$  = minimum distance between the leads of  $M_v$  and  $M_h$ .

C. Equal amplitude lines for the source of a spike potential recorded with 1.5 mm long surfaces. Each line illustrates the location of the source for which the action potential has the amplitude indicated on the line (calculated from BUCHTHAL, GULD and ROSENFALCK 1957, Table 1).

Lead 1 or 12 (Fig. 1 A) was used as *indifferent electrode*. They were situated outside the range within which potentials were recorded from the motor unit under investigation as ascertained by the absence of a response when leading off between lead 1 and lead 12. When the indifferent electrode was within the motor unit, action potentials appeared on all the leads of the multielectrode (cf. BUCHTHAL, GULD and ROSENFALCK 1957).

The *electrode noise* was reduced by passing a one to two ma current through each lead in saline for about ten seconds; it was

then about the same as that of the amplifier alone, *i. e.*  $1.5 \mu\text{V}$  r.m.s. (frequency range 2 to  $10^4$  c.p.s.).

*Pick-up range of the leading-off surfaces.*

To correlate the distribution of action potentials from a given motor unit with that of its muscle fibres, the area was determined within which active fibres can give rise to potentials on a multielectrode lead. In a previous study the decrease in spike amplitude with the distance from the potential source was measured with electrodes of  $50 \mu$  diameter up to a distance of 0.75 mm from the source (BUCHTHAL, GULD and ROSENFALCK 1957). The logarithm of spike amplitude decreased rectilinearly with the logarithm of distance. At larger distances the individual spike could no longer be discriminated with certainty on account of superimposed activity from other fibre groups of the motor unit. It seemed, however, justified to assume that the amplitude-distance relationship continued to be linear on logarithmic scales. The potential amplitude arising from a spike source at different distances from a 1.5 mm long lead was then calculated as the average of the different amplitudes along such a lead (Fig. 1 C, Table 1). The maximum spike amplitude recorded with  $50 \mu$  electrodes averaged 3.3 mV. If a potential source which gave rise to this amplitude on a  $50 \mu$  electrode were situated in immediate contact with the midpoint of the long leading-off surface an amplitude of  $780 \mu\text{V}$  would be obtained. The different curves (equal amplitude lines) in Fig. 1 C give the locations of the source whose action potential has the amplitude indicated on the curves. With the potential source at a distance of one mm from the leading-off surface the amplitude would be less than  $50 \mu\text{V}$  (Fig. 1 C). At larger distances from the potential source the amplitudes picked up with long leading-off surfaces were nearly the same as those obtained with the  $50 \mu$  electrodes, since the spike amplitudes here varied only slightly along the long lead. The spike amplitudes extrapolated for large distances are given in Table 1.

Table 1.

*Amplitude-distance relationship of a motor unit spike extrapolated<sup>1</sup> to large distances from the source.*

Distance between spike source and midpoint of the leading- off surface mm	Amplitude in $\mu\text{V}$
2	15
4	3.5
6	1.5
8	0.8
10	0.5

<sup>1</sup> for spikes with maximum amplitude above 5 mV; from Fig. 9 in BUCHTHAL, GULD and ROSENFALCK (1957).

# *Determination of the position of the multielectrodes.*

To delineate the motor units in two directions of the muscle cross section two multielectrodes were inserted perpendicularly to each other. The first multielectrode ( $M_h$ , Fig. 1 A) was inserted to the position at which maximum action potential amplitude was recorded on leads 5 to 7. The second multielectrode ( $M_r$ , Fig. 1 A) was then directed as close as possible to the first multielectrode with the centre leads opposite to those on  $M_h$  with maximum response. The electrodes were turned around their longitudinal axes until maximum amplitude of the potentials was found indicating that the leading-off surfaces faced the active muscle fibres (Fig. 1 A). In this position the two sets of leading-off surfaces were at a distance ( $d$ ) of about one mm from each other (Fig. 1 B). This accounted for a deviation from simultaneity of 0.2 msec on the two multielectrodes (propagation velocity 4.7 m per sec, BUCHTHAL, GULD and ROSENFALCK 1955b).

To determine the mutual position of the two multielectrodes subthreshold rectangular current pulses were applied through adjacent leads of one multielectrode. The field produced in the muscle was picked up by adjacent leads on the other multielectrode and the position of the multielectrodes was obtained from an analysis of this field. The pulses were taken from a stimulator via a doubly screened transformer (BUCHTHAL, GULD and ROSENFALCK 1955a). To insure rectangular current pulses two resistances (each  $10^8$  ohm) were inserted in the leads between secondary coil and electrode. On account of the capacity of the muscle fibre membranes the recorded voltage rose gradually (Fig. 2) and a pulse duration of 5 msec was required for it to reach maximum amplitude. With bipolar recording the recorded signal reversed its sign when switching from a recording lead pair on one side ( $M_r$ , leads 5 and 6, Figs. 1 and 2) to a lead pair on the other side ( $M_r$ , leads 6 and 7, Figs. 1 and 2) of the multielectrode used for signaling ( $M_s$ ). The position of the signaling electrode ( $M_h$ ) relative to the leads of the recording electrode ( $M_r$ ) was then obtained by interpolation as the point at which the signal would be zero. Conversely, the position of multielectrode  $M_r$  relative to the leads of  $M_h$  was obtained by reversing the procedure and using  $M_h$  for recording and  $M_r$  for signaling. To increase the accuracy the procedure was repeated by signaling through additional lead pairs (e. g. leads 4 and 5 and leads 6 and 7, Fig. 1 A).

In model experiments we have compared the directly with the electrically determined mutual position of the two multielectrodes. The two electrodes were mounted on micrometer sledges and placed in a large saline container. When the current signal passed through the lead pair close to the recording electrode the electrically determined position lay 0.25 mm from the line along which the leads of the signaling electrode were situated and closer to its axis. With increasing distance between signaling leads and recording electrode the displacement of the point for zero response increased up to 0.75 mm at a distance of 8 to 10 mm. This deviation from the expected position was due to a field distortion caused by the presence of the cannula of the multielectrode. It was not

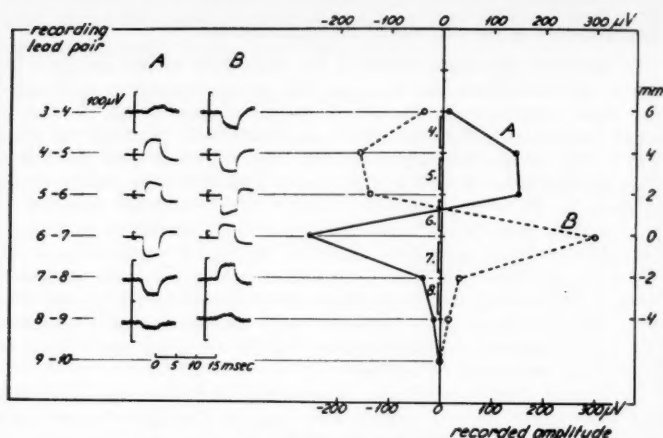


Fig. 2. To determine multielectrode position.

*Left:* voltage pulses recorded by adjacent leads on one multielectrode ( $M_v$ , Fig. 1 A) resulting from rectangular current signals through adjacent leads of the other multielectrode ( $M_h$ , Fig. 1 A) (current signals in A through leads 5-6; in B through leads 6-7).

*Right:* plot of potential amplitudes for A and B. The recorded pulse reversed its sign on lead 6. The inversion point determines the level of the leads of the signaling electrode. Correcting for the field distortion caused by the multielectrode (see text) the actual position of this level is 0.25 mm from the inversion point in the direction of lead 7.

caused by a too low common mode rejection ratio of the amplifier plus electrodes, since the rejection ratio was more than one hundred determined with the electrode inserted into the muscle (BUCHTHAL, GULD and ROSENFALCK 1954). In muscle it was not possible directly to measure the position of the inversion point relative to the multielectrode. However, the position of the inversion point varied also in muscle systematically according to the position of the signaling leads relative to the recording electrode, the total variation being 0.5 to 1 mm, *i. e.* of the same order of magnitude as in the model experiments. It seemed therefore justified to correct for the deviation in muscle by the values obtained in the model experiments. Since the lead pairs used for signaling were situated close to the recording electrode the correction applied was 0.25 mm. In this way the mutual position of the two multielectrodes could be determined with an accuracy of about 0.2 mm (Fig. 2).

## Results.

### *The spread of action potentials from a motor unit.*

If a multielectrode was randomly inserted into the muscle, action potentials from the same motor unit were recorded by up

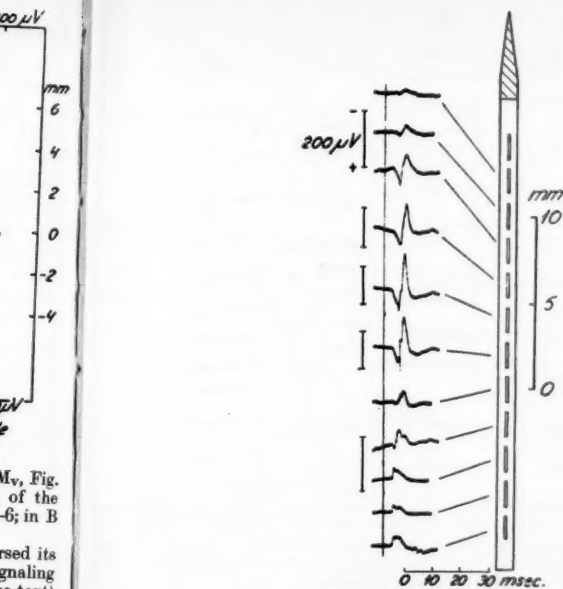


Fig. 3. Action potentials from a motor unit in the human brachial biceps recorded by eleven leads of a multielectrode with the lead nearest the tip as indifferent electrode.

to eleven adjacent leads covering 20 mm. In spite of differences in shape, amplitude and duration, these potentials could be identified as belonging to the same motor unit because of their identical time relationships whatever the frequency of discharge. Clear spike components with an amplitude of more than  $50 \mu V$  were found on only two to five adjacent leads (Fig. 3). The potentials recorded on other leads of the multielectrode consisted of low amplitude negative deflections without spikes and with a shorter duration than the potentials containing spike components. With few exceptions, the action potential amplitude as a function of the distance along the multielectrode showed a single maximum only (Figs. 4 and 5).

When two multielectrodes inserted perpendicularly to each other (see: Method p. 87) traversed the region where spike potentials were recorded from the motor unit, they appeared on approximately the same number of leads on both multielectrodes

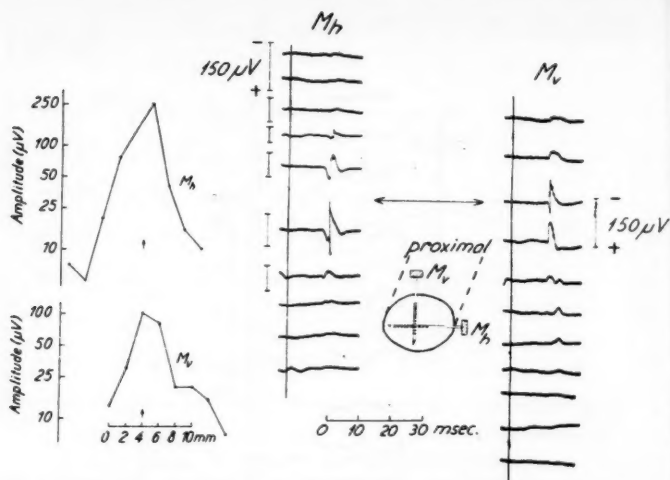


Fig. 4. *Right*: Action potentials from a motor unit in the human brachial biceps recorded along two multi-electrodes,  $M_h$ ,  $M_v$  which both traversed through the region of the motor unit in which spike potentials were recorded. The inset between the action potential columns indicates the position of the electrodes in the cross section of the muscle.

*Left*: Peak-to-peak amplitude of the action potentials (logarithmic scale) along the multi-electrodes. The arrows to the right and to the left indicate the potentials picked up by the adjacent leads of  $M_h$  and  $M_v$ .

(Fig. 4). If the second multi-electrode traversed leads on the first multi-electrode which did not record spikes, the second multi-electrode either did not pick up any potentials at all, or recorded only low amplitude potentials and on fewer leads than on the first multi-electrode (Fig. 5). The amplitude of these potentials was the lower the larger the distance of the second multi-electrode from the leads with spike responses on the first multi-electrode. These findings indicate that the action potentials from a motor unit are recorded within an area of approximately circular shape. Confirmatory evidence was obtained in a special series of experiments in which a third multi-electrode was placed parallel to one of the others and in the same cross section. When two of the multi-electrodes passed through the centre of the motor unit, potentials were recorded on more of their leads than on the third electrode.

The mean distribution of action potential amplitudes from recordings in which the horizontally and the vertically inserted

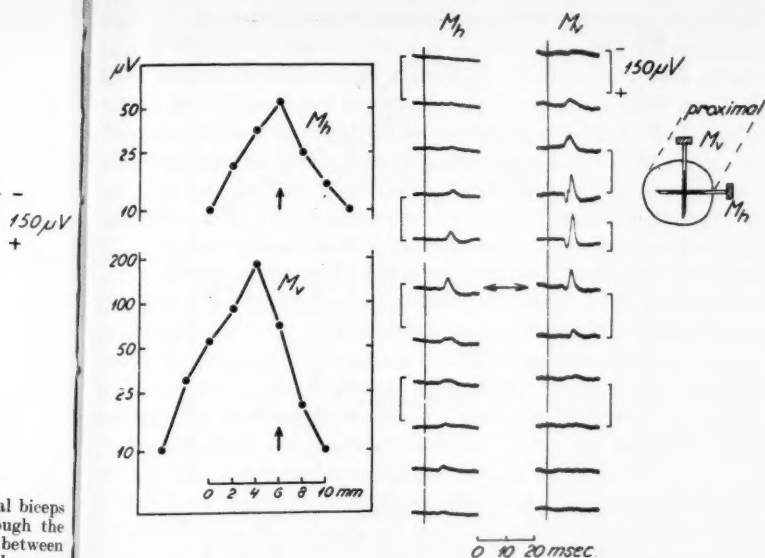


Fig. 5. *Right:* Action potentials from a motor unit in the human brachial biceps recorded along the multi-electrodes  $M_h$  and  $M_v$ .  $M_v$  traversed through the region of the motor unit in which spike potentials were recorded and  $M_h$  was situated outside this region. The inset to the right indicates the position of the electrodes in the cross section of the muscle.

*Left:* Peak-to-peak amplitude of the action potential (logarithmic scale) along the multi-electrodes. The arrows to the right and to the left indicate the potentials picked up by the adjacent leads of  $M_h$  and  $M_v$ .

multi-electrodes passed through the centre of the motor unit is given in Fig. 6 A. Within two mm from the lead with maximum response the action potential amplitude decreased by 40 or 60 per cent. Outside this range it decreased on an average proportionally to  $r^{-2.5}$ ,  $r$  being the distance along the multi-electrode from the lead with maximum response.

#### *Correlation between the spread of action potentials and the distribution of fibres of a motor unit.*

The presence of spikes in a motor unit potential with an amplitude exceeding 50  $\mu V$  indicated that the muscle fibres giving rise to these spikes were situated within less than one mm from the recording lead (Fig. 1 C). Potentials of low amplitude without spikes were volume conducted from fibres located at larger distances.

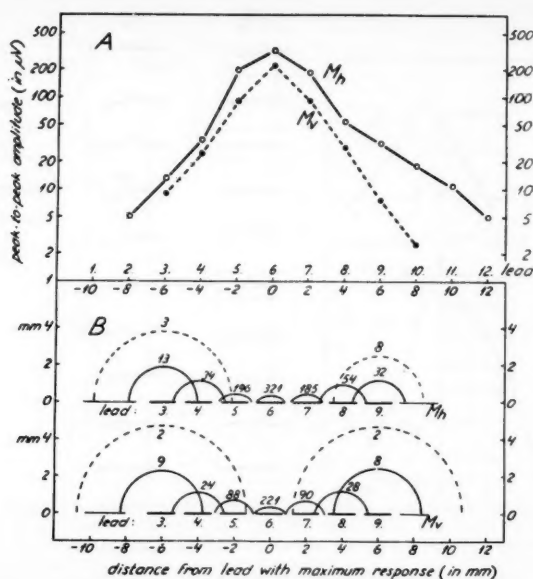


Fig. 6. A. Action potential amplitude picked up from a motor unit by the different leads of two multielectrodes inserted latero-medially ( $M_h$ ) and antero-posteriorly ( $M_v$ ) through the centre of the motor unit. Averaged from eleven motor units in the brachial biceps.

B. Equal amplitude lines indicating the position in which a spike potential source produces the action potential amplitudes in A along  $M_h$  and  $M_v$ .

The figures on the segments denote amplitude in  $\mu V$ . In leads 3 and 9 more than one spike contributed to the potential. The equal amplitude line for one of these spikes is given by the broken segment; it accounts for one fourth of the amplitudes recorded on leads 3 and 9. It is seen that all potentials may originate in the circular area with a diameter determined by leads 5–7.

ces from the recording electrode (Table 1). Therefore, the area over which action potentials could be picked up from a motor unit exceeded that over which its fibres were distributed. When this was taken into account, the extent of the motor unit could be determined. On the leads which recorded potentials of high amplitude the peak-to-peak amplitude of the motor unit potential was determined by the amplitude of one or two spikes (Figs. 3, 4, 5). The possible location of the fibre group giving rise to a given spike amplitude could be determined from the equal amplitude lines of Fig. 1 C. The possible position of the fibre group producing the amplitudes (Fig. 6 A) recorded on the different

leads of the multielectrode are indicated by the equal amplitude lines in Fig. 6 B. The amplitudes picked up by the three central leads of each multielectrode (leads 5, 6, 7) arose from fibre groups within a distance of 0.3 to 0.65 mm from the respective lead. Volume conduction of the spike potentials arising in the circular area around the three central leads of each multielectrode can account for the low amplitude potentials recorded outside this area (Fig. 6 B). The amplitude of the low potentials, though ten times higher than if derived from a single spike generated in the centre of the motor unit, decreased with increasing distance from the lead with maximum response in the same way as that of a single spike ( $r^{-2.4}$ , cf. p. 91 and BUCHTHAL, GULD and ROSENFALCK 1957). A summation of volume conducted potentials from several spike sources situated around the central leads could therefore account for the amplitudes on the peripheral leads of the multielectrodes. Such a summation is indicated by the complex shape of the low amplitude potentials (Figs. 3, 4, 5).

It follows from these considerations that the fibres of a motor unit in the brachial biceps are confined to the area within which spikes are recorded. This region had an average diameter of four to six mm. The region in which spike potentials were recorded and the spread of the action potentials of the motor unit was slightly larger in the latero-medial than in the antero-posterior direction (Fig. 6 A) indicating that the fibres belonging to a motor unit were concentrated in an oval area. The territory of different motor units varied considerably as shown by spike distributions over areas of two to ten mm in diameter. Exceptionally the distribution curve of action potential amplitudes had two maxima indicating that the fibres of the motor unit were assembled in two areas.

*Temporally dispersed spikes and total duration of the motor unit potential.*

Several spikes could be recorded within a given motor unit. On the same lead of the multielectrode 2 to 4 spikes occurred distributed over an interval of 2—3 msec (Fig. 3). The time displacement of the spikes picked up by the different leads of the two multielectrodes was larger than on the single lead and is illustrated by the examples shown in Fig. 7. The propagation velocity for different spikes was nearly identical (4.7 m per sec). Therefore, the time displacement for different spikes was mainly an expres-

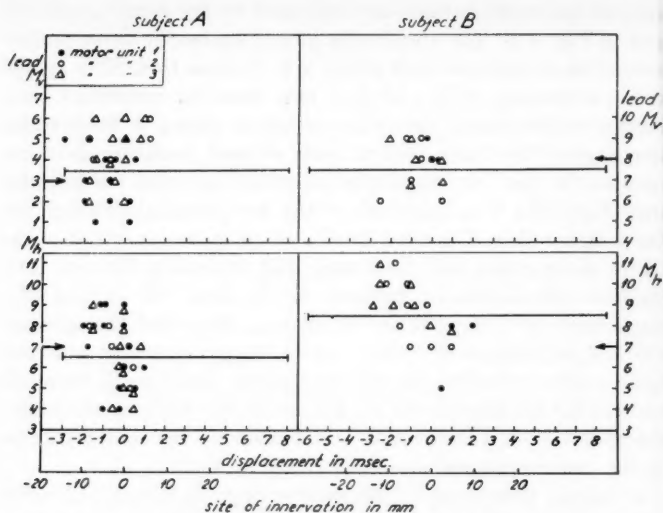


Fig. 7. Temporal dispersion of the different spikes of three overlapping motor units ( $\bullet$ ,  $\circ$ ,  $\triangle$ ) led off with two multielectrodes ( $M_v$ ,  $M_h$ ) inserted perpendicularly to each other. The onset and termination of the motor unit potential picked up from the central region of the unit is indicated by the end marks on the horizontal lines.

For subject A the electrodes were inserted in the innervation zone and for B 15–25 mm outside the zone. The arrows indicate the leads on the two multielectrodes which were closest to each other. The spread in the site of innervation (lower abscissa) was calculated from the temporal dispersion and from a propagation velocity of 4.7 m per sec.

sion of the extent of the innervation zone, a time displacement of 1 msec corresponding to 4.7 mm. The maximum time displacement for spikes within the same motor unit was five to seven msec corresponding to an extent of the innervation zone of 23 to 33 mm, confirming previous findings with a different procedure (BUCHTHAL, GULD and ROSENFALCK 1955b).

*Total duration* of a motor unit potential was defined as the time interval over which the potential could be discriminated above the noise level of the recording device. Initial and terminal *low amplitude components* were thus included in the measurements. They were present in the potentials recorded on the leads situated in the centre of the motor unit (Figs. 3, 4). All spikes of the motor unit were confined to a time interval considerably shorter than the total duration of the potentials, and we did not find spikes

simultaneous with the initial and terminal low amplitude components. This was the case even in experiments in which the entire cross section of the motor unit was searched by multiple insertions of one of the multielectrodes. Thus, there was no evidence that the initial and terminal low amplitude components derived by volume conduction from spikes simultaneous with them lying at a distance. The *initial positive component* was absent when recording from the site of innervation (Subject A, Fig. 7, Example b, Fig. 9) and its duration increased with increasing distance from the site of innervation (BUCHTHAL, GULD and ROSENFALCK 1955b). With the electrodes situated 15–25 mm outside the innervation zone the initial positive deflection began three to five msec prior to the earliest recorded spike (Subject B, Fig. 7, Example a, Fig. 9). The *protracted terminal deflection* of the motor unit potential had an amplitude of 20 to 40  $\mu$ V and could either be positive or negative (Figs. 3, 4, 5). It ended 6 to 8 msec after the latest spike recorded within the motor unit (Fig. 7).

The duration of potentials measured in different points of the same motor unit varied over the cross section of the unit. The maximal variation in duration within the region of the motor unit where spikes were recorded averaged 2.7 msec (mean duration 12 msec, 30 different motor units). This was half the variation found for all the potentials from the same thirty motor units (variation 5.1 msec).

#### *Overlapping motor units.*

Potentials from as many as six different motor units were recorded at weak or moderate effort during a two to three hour period at a given lead of the multielectrode. That the potentials belonged to different motor units was indicated by their different frequencies when they were recorded on the same sweeps at some time during the experiment (Fig. 9). The different motor units studied with a given placement of the two multielectrodes displayed the following similarities:

The action potentials of two or three of them showed a similar distribution of amplitudes along the multielectrodes (Fig. 8). If the leads of one multielectrode traversed the region of maximum amplitude for one of the motor units and if the second multielectrode traversed a region of this motor unit with potentials of low amplitude, one or two other motor units again showed a similar

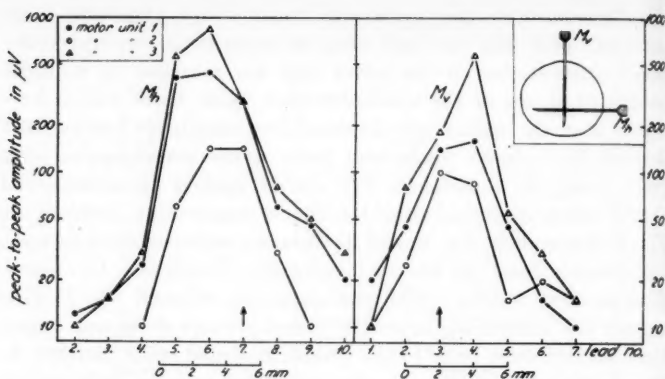


Fig. 8. Action potential amplitude recorded from three overlapping units along two multielectrodes ( $M_h$  and  $M_v$ ) inserted perpendicularly to the axis of the brachial biceps (position indicated by right inset). The arrows give the leads of  $M_h$  and  $M_v$  which were closest to each other.

distribution of action potential amplitudes. These findings indicate that two or three of the six motor units which overlap, overlap entirely, so that their muscle fibres are assembled within the same region of the cross section of the muscle.

On each multielectrode lead the different spikes of all the overlapping units scattered over similar time intervals (Fig. 7). Since the spread of arrival times of different spikes reflects the spread of the site of the motor end plates, this finding demonstrates an identical breadth though not necessarily an identical situation of the end plate zone of the overlapping motor units. The potentials recorded from different motor units with a given lead of the multielectrode frequently showed a striking similarity in shape (Fig. 9). This applied to the occurrence of different spike components and other minor irregularities. The similarity in shape shows that the temporal dispersion of the spikes was identical within each of the overlapping motor units. In addition it indicates that the fibre groups giving rise to corresponding spikes within the potentials of these overlapping units were situated at nearly identical distances from the recording lead. In terms of innervation zone this finding suggests that the site of innervation of adjacent fibre groups from overlapping units is situated in very nearly the same cross section of the muscle. That this was in fact the case was evidenced by experiments in which the electrode was situated

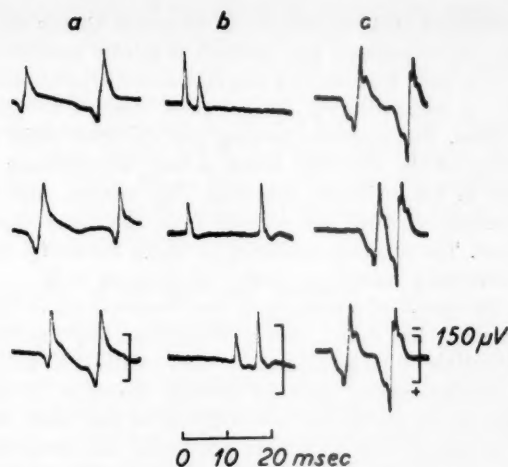


Fig. 9. Similarity in shape of action potentials from different motor units recorded on the same lead and identified by different frequency. To show the different frequency the three examples (a, b, c) are each given with three different time intervals.

within the innervation zone. There the action potentials of two overlapping motor units frequently started with an abrupt negative deflection (Fig. 9 b), indicating that the recording lead for both motor units was in the immediate vicinity of the site of initiation of a spike potential (BUCHTHAL, GULD and ROSENFALCK 1955b). In other words, the nerve supply to the fibre groups from overlapping motor units runs parallel even in the most distal ramifications.

### Discussion.

On account of the volume conductor which surrounds the fibres one must expect action potentials to be picked up from all points within and somewhat outside the area in which the fibres of the motor unit are located independent of whether there are active fibres at these points or not. In the present multielectrode study the distribution of these potentials was investigated and analysed with respect to the territory covered by the fibres of a motor unit. In previous investigations displacement of single electrodes was used for this purpose (DENSLOW and HASSETT 1943, DENSLOW

and GUTENSOHN 1950, DENSLOW, GUTENSOHN, CHASE and KUMM 1956). In our experience this method is hardly suitable to give quantitative data: frequently a displacement of the electrode does not cause a corresponding displacement relative to the active muscle fibres. Furthermore, moving one electrode often changes the position of the electrode which is used as reference; thereby the shape of the reference potential may change, and it is no longer certain whether one records from the same motor unit throughout. The striking similarity in shape shown by responses from overlapping units is a source of error as well.

As to the spread of potentials in the *transverse* plane our multi-electrode study has shown that in the human brachial biceps the spike potentials of each motor unit were localised to an approximately circular region, with an average diameter of five mm. The fibres of the motor unit are confined to this area. Including volume conducted low-amplitude potentials the motor unit was traced over up to 20 mm. A spread of the motor unit potential over up to 60 mm in the transverse direction has been described in the brachial biceps (DENSLOW and HASSETT 1943). This might be due to pick up by the indifferent electrode (cf. p. 85). The spread over up to 70 mm in the *longitudinal* direction (DENSLOW and HASSETT 1943) was to be expected since in the brachial biceps muscle fibres pass uninterruptedly through the muscle (BUCHTHAL, GULD and ROSENFALCK 1955a).

The area over which the fibres of a motor unit are spread is larger than would correspond to a close packing of its fibres. Direct measurements of the innervation ratio in the human brachial biceps are not available. From FEINSTEIN, LINDEGÅRD, NYMAN and WOHLFART's (1955) figures in other human muscles and from an indirect evaluation for the brachial biceps (BUCHTHAL and MADSEN 1950), 1,000 muscle fibres per motor unit seems a reasonable assumption. With a mean fibre diameter of 50  $\mu$  (SCHWALBE and MAYEDA 1890, BUCHTHAL, GULD and ROSENFALCK 1955a) an area of 5 mm diameter contains 10,000 muscle fibres. Hence, an average of ten motor units should be situated within the area within which the fibres of the single motor unit are distributed. In fact, up to six different motor units were found even at weak and moderate effort on the same lead. Histological evidence for this overlap in the rabbit has been presented for sartorius (VAN HARREVELD 1946) and the small digital muscles (FEINDEL, HINSHAW and WEDDELL 1952, FEINDEL 1954).

The intermingling of the fibres of overlapping motor units is not random. Electrophysiological and histological evidence presented in a previous study showed the fibres of each motor unit to be assembled in small groups with maximally thirty fibres, their mutual distance was 0.3 to 1.0 mm and the temporal dispersion of their spikes 0.5 to 2.3 msec (BUCHTHAL, GULD and ROSENFALCK 1957). These fibre groups are the sources of the spatially and temporally dispersed spikes recorded at different points in the motor unit.

*The parameters of the motor unit potential.*

In previous and in the present studies information was gathered as to the distribution of the fibres of a motor unit, the site of their innervation, the conduction of the action potential over the fibres and the volume conduction of the spike potential within the muscle (BUCHTHAL, GULD and ROSENFALCK 1955a,b, 1957). This evidence may elucidate many previously described features of the motor unit potential as recorded with randomly inserted electrodes in the muscle. Particularly an attempt will be made to account for the large variation in amplitude, shape and duration (BUCHTHAL, GULD and ROSENFALCK 1954).

The amplitude of the motor unit potential when it contains spike components is determined by one or two spikes. These originate from small groups of muscle fibres (subunits) separated by fibres from other intermingling motor units and at a distance of 0.3 to 1 mm from each other. The marked decrease in spike amplitude with the distance from the spike source accounts for the wide variation in amplitude found at random insertion of an electrode. With a concentric electrode (leading-off area  $0.1 \times 0.35$  mm) situated within the area of the fibres of a motor unit the distance to the nearest spike source may vary between a few and  $500 \mu$  resulting in a variation in amplitudes from several millivolts to  $150 \mu$ V. If the electrode is situated outside the area of the fibres of the motor unit only low amplitude potentials without spikes are recorded. They represent the summated volume conducted potentials from the spike sources of the motor unit (cf. p. 93). The potential amplitude depends also on the size of the leading-off area of the electrode. The electrode records the average of the potential along its leading-off surface. Therefore, the maximum amplitude which is recorded increases with decreasing leading-off area until the electrode reaches a diameter which is small in

comparison to the diameter of the spike source (HÅKANSSON 1956).

The *total duration of the motor unit potential* depends on the site of the electrode relative to the fibres of the activated motor unit. If the electrode is situated within the region where fibres are located the potential has a longer duration than the volume conducted potentials recorded outside the motor unit. On account of the summation of temporally dispersed action potentials from different muscle fibres the total duration of the motor unit potential exceeds that of the single fibre potential. The temporal dispersion can only to a minor degree be explained by differences in propagation velocity of the different fibres. It results mainly from the spatial dispersion of sites of innervation. It could in fact be demonstrated by the simultaneous abrupt start of the potentials in different points of a motor unit that the sites of innervation are scattered over 20–30 mm along the length of the muscle fibres of the same motor unit. This can account for differences in arrival time of at most 7 msec (propagation velocity 4.7 m per sec). With a duration of the single fibre potential (fibrillation potential) of 2 to 3 msec total durations of up to 10 msec may thus be accounted for. Within the innervation zone the temporal dispersion is less than outside this zone and hence the action potential duration is shorter. To explain the protracted terminal low amplitude components, it is, however, necessary to assume that the single fibre potentials in fact have a longer duration than they appear to have when recorded as fibrillation potentials. This assumption is supported by the finding of a repolarisation for intracellularly recorded action potentials in mammalian muscle fibres, which may last 7 msec (TRAUTWEIN, ZINK and KAYSER 1953). The motor unit potential represents the summated activity of many neighbouring fibres, and protracted components of their potentials which disappear in the noise level when recording outside a single fibre may add up to appear in the composed potential. The initial and terminal low amplitude components decrease relatively less with the transverse distance from the source than the spike potential (cf. the shorter duration with bipolar recording). Thus the inactive fibres seem to represent a higher impedance for slow than for faster potential changes. This would facilitate an electrotonic spread along the fibres of the currents from a depolarised region. It is in agreement with this assumption that the initial positive wave in the motor unit poten-

tial is most pronounced near the site of innervation and that its duration is the longer the larger the distance along the fibres between electrode and site of innervation.

The *shape of the motor unit potential* results from the summation of spatially and temporally dispersed action potentials from the spike sources of the motor unit. On account of the marked decrease in amplitude with increasing distance only two to three of the fibre groups determine the shape of the motor unit potential. A slight temporal dispersion of the component spikes causes di- or triphasic potentials with "knots" and "humps" ('irregular potentials'). Potentials with four or more phases derive from summation of spike potentials with a temporal dispersion of several milliseconds and with the spike sources at about equal distances from the recording electrode. The shape of the motor unit potential depends also on the position of the electrode relative to the innervation zone: The potential starts with a steep negative deflection if the electrode is situated in the immediate vicinity of the end plates.

The parameters of the motor unit potential depend as well on the *type of electrode*. With bipolar recording the duration is shorter than with unipolar or concentric recording. This reduction is due to the absence of initial and terminal low amplitude components in the potential. These low amplitude potentials have about the same amplitude on both leading-off surfaces of the bipolar electrode and disappear therefore with measurement of the potential difference. With an interelectrode distance of 0.5 mm the spike amplitude is about the same with bipolar as with concentric recording. With smaller interelectrode distances the spike amplitude is reduced since the same spike appears on both leading-off surfaces with about the same amplitude (PETERSÉN and KUGELBERG 1949, LUNDERVOLD and CHOH-LUH-LI 1953).

### Summary.

By means of multielectrodes containing twelve leads along a 25 mm distance (Fig. 1) the spread of action potentials from a motor unit was studied over a cross section of the brachial biceps in man. The territory of the motor unit could be determined from the analysis of a single cross section of the muscle, since its fibres pass uninterrupted from tendon to tendon.

The mutual position of two multielectrodes inserted perpendicularly to each other into the muscle was determined with an accuracy of 0.2 mm by recording with one multielectrode subthreshold pulses sent through successive leads of the other multielectrode (Fig. 2).

The action potentials of a motor unit spread over an approximately circular area as evidenced by experiments in which the two multielectrodes traversed the centre of the motor unit (Fig. 4) and other experiments in which one of the multielectrodes was situated at the periphery of the motor unit (Fig. 5).

Potentials with spike components exceeding 50  $\mu$ V were confined to an average area four to six mm in diameter (Fig. 6 A). These potentials had a protracted terminal component of low amplitude and if they were recorded outside the site of innervation an initial positive component as well. Outside the area in which spike potentials occurred the potentials had a low amplitude, a short duration and a negative sign (Figs. 3, 4, 5).

On account of volume conduction the action potentials were picked up in a larger area than occupied by the fibres of the motor unit. By means of the relationship between spike amplitude and distance from the potential source (Fig. 1 C) the average territory of the motor unit was determined to be four to six mm (minimum two and maximum ten mm) (Fig. 6 B). This area allows space for the fibres of ten overlapping motor units.

On the same lead of the multielectrode action potentials from maximally six different motor units were recorded. Their potentials were often similar in shape so that they could be identified only by their discharge frequency (Fig. 9). The muscle fibres of as many as three of the intermingling motor units overlapped entirely (Fig. 8). The temporal dispersion of the spikes recorded on the different leads of the multielectrode was identical for these overlapping motor units (Fig. 7).

The results are discussed with respect to their bearing on the parameters of the motor unit potential: The action potential of the motor unit arises from the summation of temporally dispersed potentials originating from fibre groups at a mutual distance of 0.3 to 1 mm. The amplitude of the motor unit potential is largely determined by the distance of the recording electrode from the fibre group closest to the electrode. The shape of the potential derives from the way in which the action potentials of two to three of these fibre groups summate. The duration of the potential

is determined by the temporal dispersion of the component potentials due to the spatial dispersion of the end plates in the innervation zone.

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